ADVANCES IN PLANT MORPHOLOGY

(PROFESSOR V. PURI COMMEMORATION VOLUME)

Editors

Y. S. MURTY

B. M. JOHRI

H. Y. MOHAN RAM

T. M. VARGHESE

SARITA PRAKASHAN
NAUCHANDI GROUNDS, MEERUT. (INDIA)

PROFESSOR V. PURI COMMEMORATION ORGANIZING COMMITTEE

Chairman	*Patron*	***Convenor***
PROFESSOR B. M. JOHRI University of Delhi	PROFESSOR J. N. KAPUR Meerut University	**PROFESSOR Y. S. MURTY** **Meerut University**

Members

PROFESSOR H. Y. MOHAN RAM University of Delhi	PROFESSOR R. KHAN Aligarh Muslim University
PROFESSOR B. TIAGI Rajasthan University, Jaipur	PROFESSOR Y. D. TIAGI University of Udaipur

581.4 P
M [illegible]

Cat

PUBLISHED BY SMT. SAVITRI RASTOGI FOR SARITA PRAKASHAN AND
PRINTED BY K. A. RASTOGI AT THE PRABHAT PRESS, MEERUT, U. P. INDIA

Professor [illegible]. Puri

PROFESSOR V. PURI : BIOGRAPHIC SKETCH

Born on December 9, 1909. at Nagina U.P., Vishwambhar Puri had his schooling at Meerut. He did High School in 1928 from the Dev Nagri School (now College) where he came under the powerful influence of such devoted teachers as Pt. G.D. Pandey (Head Master) Mr. Ugra Sen, Mr. Jagan Nath Prasad, etc. For college education he shifted to Agra from where he did B. Sc. (1932) and M. Sc. (1934) both with credit. He was awarded the Lord Reading prize for being the best all round M.Sc. student during 1933-34. Professor P. Maheshwari had collected a team of devoted research students interested in plant morphology and Puri was naturally drawn to it. He was the third person to receive the D.Sc. degree of the Agra University in 1940, after completing his work in just five years, the minimum time requirement of the University under the guidance of Dr. Panchanan Maheshwari. That he could do this while also teaching and building up a department in the Meerut College speaks well of his capacity for hard, consistent and methodical work.

Professor Puri's professional career started in Meerut in 1935 when he was appointed a lecturer in Meerut College to teach Intermediate classes in biology, started that year only. When he was selected for this post from among many bright and qualified candidates, he was conveyed the good news by his guardian Dr. Bhupal Singh (the then Secretary of the College) in words which he says he could never forget. This eminent surgeon, a great disciplinarian, with exceptionally high professional ethics spoke to young Vishwambhar in a grave tone and said "You have been selected for the post, *but....*" and he left the sentence incomplete. This 'but' says Professor Puri, remained for him a word of warning, of challenge, of a promise of fulfilment that he silently made to himself and his benefactor.

Professor Puri spent his life time in building up this department and raising it from an Intermediate status to B.Sc. and M.Sc. levels. It was a research department from the beginning in the sense that he did all his research work here.

He visited Cornell University during 1949-1950, and collaborated with the late Professor A. J. Eames. During this visit he established contacts with Professional colleagues and poured over valuable literature available in the libraries of U.S.A. These resulted in the preparation of two outstanding reviews on 'Floral Anatomy' and 'Placentation'.

Immediately on his return to India, he became deeply interested in establishing a 'School of Plant Morphology'. He had the resourcefulness to tap several granting bodies to accomplish the task, and the full support of principal Madan Mohan of Meerut College. The foundation stone of the building of the proposed School was laid by late Dr. S. S. Bhatnagar FRS in 1953. By next year the building was ready for use. The school soon became an active centre of research, and attracted a large number of dedicated workers of whom over 24 have already received their doctorate degree. Professor Puri was able to establish an excellent library in the school that could be the envy of any University.

A number of persons, who later occupied eminent positions in the country worked in this department for varying periods. Some of these are S.P. Capoor. (Professor, Government Agri. College, Poona), M.L. Gattani (Advisor, UNESCO), B.M. Johri (Professor, University of Delhi), U.N. Chatterji (Professor. University of Jodhpur), L.B. Kajale (Professor, University of Nagpur), P.D. Gupta, (Professor, University of Lucknow) S.K. Jain (Deputy Director Botanical Survey of India), S.N. Mathur (Professor, Tribhuvan University, Kathmandu, Nepal), Y.S. Murty (Professor, Meerut University), etc. All these years Professor Puri developed so much interest and attachment to this department that he declined some good offers from other universities.

Long respected for his abundant energy, liberality of outlook, and a high sense of values, Professor Puri was appointed Principal of Meerut College in 1965. When the Meerut University was established in 1969, he was offered the chair of Botany in the Institute of Advanced Studies.

Since his retirement in June 1972 as Senior Professor and Head of the Department of Botany, he is working as Professor Emeritus in the University providing leadership in research and programmes of teaching improvement. He is the Director of the U.G.C. University Leadership Project for this University.

Ever since Puri joined Meerut College he was actively engaged in research but not at the cost of teaching which he considered to be more important for a teacher. Addressing the section of Biological sciences of the National Academy of Science at Ahmedabad in February 1968, he made a strong plea for treating good teachers at par with research specialists in the following words: ". . . . their activities should be regarded as scholarly, or intellectually respectable, as those of the latter. In fact teaching and teaching correctly and adequately—is more basic than research. A stage has now come when we have to decide whether for specific jobs we want teachers or investigators; scientists or technicians. And with more and more specialization this will become more and more important."

Another point that has to be emphasized is that Professor Puri never allowed his name to be appended to the work of his students. Some 18 students have so far obtained Ph.D. under his guidance and some of them are quite active and producing good work independently. Only when he has thought he had made some substantial contribution to the work did he allow his name appear as co-author.

Notwithstanding this, Professor Puri has over sixty articles and research papers to his credit published in reputed journals in India and abroad. The two review articles written in early fifties are the first of their kind in the study of vasculature of the flower and have often been referred to in subsequent works. His works on floral morphology in general and carpel structure in particular in Rhoeadales, Parietales, Cucurbitaceae and Gentianaceae and on coronal structures in Passifloraceae and Asclepiadaceae and sporocarp of *Marsilea* speak for themselves. He reviewed the work done on floral anatomy in this country and elsewhere from time to time, and discussed the taxonomic value of this branch of study.

Professor Puri made several visits abroad in pursuit of his academic interests. In April 1949 he went to U.S.A. to work at Cornell University. Ithaca, N.Y. There he studied extensively and prepared material for future study, and had very exhaustive discussion with Professors A.J. Eames, L.H. MacDaniels and H.P.

19. ——. The role of floral anatomy in the solution of morphological problems. *Bot. Rev.* **17:** 471–553. 1951.

20. ——. The flower. I. An appeal for wider interest in plants. *Agra Uni. Exten. Lecture*. 1–6. B. R. College, Agra. 1952.

21. ——. The flower. II. The origin of flower. *Agra Univ. Exten. Lecture*. 7–22. B. R. College, Agra. 1952.

22. ——. The flower. III. Placentation in the Rhoeadalean flower *Agra Univ. Exten. Lecture*. 23–36. 1952.

23. ——. Floral anatomy and inferior ovary. *Phytomorphology* **2:** 122–129. 1952.

24. ——. The Natural Philosophy of Plant Form by Agnes Arber, Review. *Phytomorphology* **2:** 186–188. 1952.

25. ——. Placentation in Angiosperms *Bot. Rev.* **18:** 603–651. 1952.

26. ——. Floral anatomy in relation to taxonomy. *Agra Univ. J. Res.* **1:** 15–35. 1952.

27. ——. 'Biomorphology in General: Schoute' Review. *Phytomorphology* **2:** 253–254. 1952.

28. ——. AND M. M. GARG. Morphology of the sporocarp in the Marsileaceae. *Curr. Sci.* **22:** 150–152. 1952.

29. ——. AND M. M. GARG. A contribution to the anatomy of the sporocarp of *Marsilea minuta* L., with a discussion of the nature of sporocarp in the Marsileaceae. *Phytomorphology* **3:** 190–208. 1953.

30. ——. 'The Mind and Eye by Agnes Arber' Review. *Phytomorphology* **4:** 256–258. 1954.

31. ——. Studies in floral anatomy. VII. Placentation in certain species of the Cucurbitaceae. *Phytomorphology* **4:** 278–299. 1954.

32. ——. On the relation between leaf and stem. *Bull. bot. Soc. Bengal* **8:** 101–106. 1954.

33. ——. History of Plant morphology. I. Lecture. *Agra Univ. Exten. Lecture* 1–9. D.A.V. College Kanpur. 1955.

34. ——. Recent advances in Plant Morphology. II. Lecture *Agra Univ. Exten. Lecture.* 10–33. D.A.V. College, Kanpur. 1955.

35. ——. "Contribution to plant anatomy by I.W. Bailey' Review. *Phytomorphology* **5:** 472–475. 1955.

36. ——. Twenty years of floral anatomy. *Uttara Bharti* **3(i):** 82–106. 1956.

37. ——Floral anatomy and taxonomy. *Indian bot. Soc. Memoire*. **1:** 15–18. 1958.

38. ——. "An atlas of airborne pollen grains by HYDE, H.A. and ADMAS, K.F." Review. *Curr. Sci.* **27:** 417–18. 1958.

39. ——. On the structure and evolution of angiosperm carpel. *Proc. IX Intern. bot. Cong.* Montreal 313–314. 1959.

40. ——. Carpel Morphology. *Agra. Univ. Exten. Lecture*. 1–27. Govt. Agri. College, Kanpur. 1960.

41. ——. "Monograph on Marsilea by Gupta, K.M." Review. *J.* 1960.

42. ——. On the methods of studying floral morphology. *Proc. Nat. Inst. Sci.* India **26:** 97–108. 1961.

43. ——. The classical concept of angiosperm carpel; A Reassessment. *J. Indian bot. Soc.* **40:** 511–524. 1961.

44. ——. *History of Botanical Researches in India, Burma* and Ceylon. Pt. IV. Floral Anatomy. 1–31. 1961.

45. ——. "Advances in morphogenesis Vol. 1; eds. Aberchrombie, M. and Brachet, Jean" Review, *Curr. Sci.* **30:** 442. **1961.**

46. ——. On the concept of carpellary margins. Proc. Summer school of Botany, Darjeeling" held June 2–15, 1960. Edited by P. Maheshwari et al. Published April. 1962.

47. ——. Morphology of Angiosperm flower. Proc. Summer School of Botany, Darjeeling" held June 2–15, 1960. Edited by P. Maheshwari et al. Published April. 1962.

48. ——. AND G. GOPAL KRISHNA. Morphology of the flower of some Gentianaceae with special reference to placentation. *Bot. Gaz.* **124:** 42–57. 1962.

49. ——. Floral anatomy in relation to taxonomy *Bull. bot. Surv. India.* **4:** 161–165. 1962.

50. ——. Classification and Phylogeny. *Bull. Bot. Surv. India.* **4:** 167–172. 1962.

51. ——. Die Evolution der Angiospermen by A. Takhtajan. Review. *Phytomorphology* **12:** 432–433. 1962.

52. ——. AND BHAMBIE. Shoot apex organisation in Lycopodiales. Mem. *Indian bot. Soc.* No. 4, 55–63. 1963.

53. ——. On the relation between ovule and carpel. Maheshwari Comn. Volume. *J. Indian bot. Soc.* **42A:** 189–198. 1964.

54. ——. The Gentianaceae and the so-called evolutionary trends. *Proc. X Intern. bot. Congress.* Abs. 142–143. Edinburgh. 1964.

55. ——. Disappointments of a morphologist. Address delivered on the occasion of presentation of Birbal Sahni Medal on Dec. 31, 1964, Calcutta. *J. Indian bot. Soc.* **44:** 7–14. 1965.

56. ——. PROFESSOR PANCHANAMAN MAHESHWARI, F. R. S. Obituary, *Sci. Rept.* **3:** 325–326. 1966.

57. ——. Telome theory as applied to higher taxa—Angiosperms. Proc. Autumn School in Botany Ed. T. S. Mahabale (Published in 1970). 1966.

58. ——. and R. Shiam, Studies in floral anatomy. VIII. Vascular Anatomy of the flower of certain species of the Asclepiadaceae with special reference to corona. *Agra Univ. J. Res.* (Sci) **15**: 189–216. 1967.

59. ——. The origin and evolution of angiosperms. Presidential address 46th session Indian bot. Soc. *J. Indian bot. Soc.* **46**: 1–14. 1967.

60. ——. In defence of morphology. Presidential address Section of Biological Sciences, 37th Annual Session. *National Acad. Sci.* India. Ahmedabad Feb. 1-4. 1968 pp. 41-48. 1968.

61. ——. Morphology: Its future. Inaugural Address in Seminar on Morphology, Anatomy and Embryology of land Plants. Delhi University Delhi, Dec. 23-28, 1969. pp. 1-4. 1969.

62. ——. Teaching of Form, Structure and Function in a co-ordinated manner. *Proc. Indian Sci. Cong.* **57**: 343. 1970.

63. ——. The angiosperm ovule. Presidential Address botany Section 57th Session Indian Sci. Cong. Kharagpur. 1970.

64. ——. "Anatomy of Seed Plants by K. Esau" Review. *Indian J. Agri. Res.* Vol. 1970.

65. ——. Anther Sacs and pollen grains: Some aspects of their structure and function. 6th Annual Lecture Indian Paly. Society. Bangalore. *J. Palynology* **6**: 1–17. 1971.

66. ——. Form, structure and Function: Their interdependence and inter-relatedness. Symposium, Botany Section, *Indian Sci. Cong.* Bangalore. 1971.

67. ——. Modernism in Biology. Symposium organised by Taraquie-Urdu Board New Delhi. 1971.

68. ——. On some basic assumption in plant morphology. Golden Jubilee Volume. *J. Indian bot. Soc.* **50 (A)**: 196–202. 1971.

PREFACE

This Volume, which contains numerous original articles and reviews on 'Advances in Plant Morphology', has been brought out on the occasion of the retirement of Professor Vishwambhar Puri from the Chair of Botany at Meerut University. Those who have been intimately associated with Professor Puri realize that retirement, in his case, must be understood only in the sense of the calendar. He has proved that being a botanist and a teacher is a way of life, rather than doing a profession.

Starting as a lecturer in biology at Meerut College. he organized a full-fledged Post-Graduate Department and, finally, the School of Plant Morphology, with excellent research and library facilities. His original and note-worthy, researches in floral anatomy have added substantial knowledge to the origin, morphology and evolution of the angiosperm flower. He has inspired and trained numerous colleagues and students to continue the work he initiated. With advancing years, his interests widened and he was compelled to devote his energies and experience to the wider aspects of education. He accepted, rather reluctantly, the arduous job of Principal of Meerut College—one of the largest in the country. Even in the establishment of the newly started Meerut University, Professor Puri's contributions have not been insignificant.

The topics presented in this Volume cover a wide range. Wardlaw critically analyses the prospect and retrospect of plant anatomy, Metcalfe discusses the descriptive writings in plant anatomy, Meeuse interprets the affinities between Piperales and Polycarpicae on the basis of floral morphology, Scheler and Banks portray the periderm characters in some Devonian plants, Fahn and Kotler trace the development of the banana flower, Tucker evaluates the ontogenetic evidence in floral morphology, and Moseley shares the thoughts of a phylogenetic anatomist on the evolution of the flower. Greyson discusses alternative goals for flower morphology, and Sattler provides evidence for centrifugal primordial inception in floral development.

Swaminathan sheds light on the genetic reconstruction of crop ideotypes, Roy and co-workers discuss speciation in relation to breeding system in cucurbits, and Guignard presents a classification of fruits. Periasamy proposes a new hypothesis of the basic mechanism of morphogenesis, Shah and co-workers have discussed the vascular supply to the axillary buds, Norstog has described the early ontogeny of the grass embryo and endosperm, Johri and Srivastava have detailed the growth responses of cultured endosperm in castor, Kapil and Bhatnagar have appraised the endosperm in Euphorbiaceae, and Venkata Rao the phylogeny of the same family. Rau gives an account of the phytogeography of some rare angiosperms of the Western Himalayas, There are morpho-taxonomical and anatomical accounts of specific taxa by Subramanyam, Bocquet and Saxena, Dnyansagar, Shah, T.A. Rao, J.K. Maheshwari, Mahabale, Nair and Tiagi. Apical organization of the root, and of the shoot during transition to flowering have been discussed by Pillai, and Murty and Kumar, respectively. The effects of ethephon on plant development have been reviewed by Mohan Ram and Jaiswal, and A.N. Rao has analysed the morphogenetic effects of growth substances on *Regnellidium*. S.C. Maheshwari projects the shape of morphogenesis in the coming decades. There are, in addition, several other valuable contributions.

The readiness with which the numerous professional colleagues responded to our request to send their contributions, and the quality of the articles reflect the high esteem in which they hold Professor Puri. It is hoped that in honouring Professor Puri, this Volume will further the frontiers of knowledge in plant morphology.

We would also like to express our sincere thanks to all those who contributed their valuable articles and offered help in various ways for the completion of this task. Dr Vishnu Kumar and Dr Sneh Lata Mathur deserve our special thanks for their help in editing. We are deeply indebted to the members of the Organizing committee for their help and cooperation in this pleasant endeavour. We would like to place on record an appreciation and thanks for the cooperation extended to us by Mr. K. A. Rastogi of Prabhat Press in connection with bringing out this volume.

EDITORS

Contents

		Page
PROFESSOR V. PURI : BIOGRAPHIC SKETCH		*v*
PREFACE		*xi*
PLANT ANATOMY IN RETROSPECT AND PROSPECT.	*Wardlaw, C. W.*	1
TAXONOMIC AFFINITIES BETWEEN PIPERALES AND POLYCARPICAE AND THEIR IMPLICATIONS IN INTERPRETATIVE FLORAL MORPHOLOGY.	*Meeuse, A. D. J.*	3
ALTERNATIVE GOALS FOR FLOWER MORPHOLOGY.	*Greyson, Richard I.*	28
CONTEMPORARY THOUGHTS IN PLANT MORPHOLOGY AND A NEW HYPOTHESIS OF THE BASIC MECHANISM OF MORPHOGENESIS.	*Periasamy, K.*	33
FOLIAR SCLEREID PATTERNS AND THEIR ORIGIN.	*Rao, T. Ananda* *Bhupal, O. P.*	46
MINOR VENATION PATTERN IN THE INDIAN ACERS.	*Banerji, M L.* *Das, Sitesh*	51
PERIDERM IN SOME DEVONIAN PLANTS.	*Scheckler, Stephen E.* *Banks, Harlan P.*	58
VASCULAR SUPPLY OF AXILLARY BUDS IN SOME DICOTYLEDONS.	*Shah, J. J.* *Patel, J D.* *Kothari, I. L.*	65
DESCRIPTIVE WRITING WITH SPECIAL REFERENCE TO PLANT ANATOMY.	*Metcalfe, C. R.*	76
FLORAL ANATOMY OF *RICINOCARPUS PINIFOLIUS* DESF. WITH SOME OBSERVATIONS ON THE PHYLOGENY AND CENTRE OF ORIGIN OF EUPHORBIACEAE.	*Rao, C. Venkata*	85
AN EXTENDED INVESTIGATION OF THE MEGAGAMETOPHYTE IN *OXALIS CORNICULATA* L.	*Herr, J. M. Jr.*	92
MORPHOLOGY AND EMBRYOLOGY OF THE FAMILY CYPERACEAE—AN APPRAISAL.	*Shah, C. K.*	102

The early development of the grass embryo and endosperm.	*Norstog, Knut*	113
Apomixis.	*Davis, Gwenda L.*	126
Recent Finds of some Rare Angiosperms in Western Himalaya and their Phytogeographic Significance.	*Rau, M. A.*	133
Morpho-taxonomic Studies on Indian Guttiferae. The Genera *Mammea* Linn S. I. and *Kayea* wall.	*Maheshwari, J. K.*	137
The Development of the Flower and Nectary of the Dwarf Cavendish Banana.	*Fahn, A.* *Kotler, M.*	153
Centrifugal Primordial Inception in Floral Development.	*Sattler, Rolf*	170
Autotetraploid of *Rauvolfia serpentina*.	*Dnyansagar, V. R.* *Torne, S. G.*	179
Speciation in relation to the Breeding System in Cucurbitaceae.	*Roy, R. P.* *Saran, Sunil* *Dutt, Bithi*	193
Genetic Reconstruction of crop Ideotypes.	*Swaminathan, M. S.*	203
Some aspects of the Floral Morphology and Embryology of *Flagellaria indica* Linn.	*Subramanyam, K.* *Narayana, H. S.*	211
Studies on Palms: Embryology of *Caryota urens* L.	*Shirke, Nandini S.* *Mahabale, T. S.*	218
Purioxylon jurassica Gen. Et Sp. Nov. from Amarjola in the Rajmahal Hills, India.	*Sharma, B. D.*	233
On the Phenomenon of Cauliflory in Angiosperms.	*Pundir, Y. P. S.*	243
Sur La Classification Des Fruits (About the Classification of Fruits).	*Guignard, Jean-Louis*	251
✓ Pollen Morphology and Phylogenetic Classification of Primitive Angiosperms.	*Nair, P. K. K.*	255

FLORAL MORPHOLOGY AND EMBRYOLOGY OF *MYRISTICA MALABARICA* LAMK. WITH A DISCUSSION ON CERTAIN ASPECTS OF THE SYSTEMATICS OF MYRISTICACEAE.	*Nair, N. C.*	264
ROOT APICAL ORGANIZATION IN VASCULAR PLANTS.	*Pillai, S. K.*	278
VEGETATIVE TO REPRODUCTIVE STATE OF APEX IN MONOCOTYLEDONS.	*Murty, Y. S.* *Kumar, V.*	291
ETHEPHON AND PLANT DEVELOPMENT : AN APPRAISAL.	*Mohan Ram, H. Y.* *Jaiswal, V. S.*	304
ROLE OF INSOLUBLE POLYSACCHARIDES IN THE ANTHER DEVELOPMENT OF SOME MEMBERS OF GRAMINEAE-MILLETS.	*Panchaksharappa, M. G.* *Rudramuniyappa, C. K.*	315
MORPHOGENETIC EFFECTS OF GROWTH SUBSTANCES ON *REGNELLIDIUM DIPHYLLUM*.	*Rao, A. N.*	325
IN VITRO GROWTH RESPONSES OF MATURE ENDOSPERM OF *RICINUS COMMUNIS* L.	*Johri, B. M.* *Srivastava, P. S.*	339
THE ROLE OF ONTOGENETIC EVIDENCE IN FLORAL MORPHOLOGY.	*Tucker, Shirley C.*	359
A NEW KIND OF ENDOSPERM IN *RHINANTHUS MAJOR* EHRH AND *R. SEROTINUS* (SCHOENL) SCHINZ AND THELL.	*Tiagi, B.*	370
ENDOSPERM IN EUPHORBIACEAE—A CRITICAL APPRAISAL.	*Kapil, R. N.* *Bhatnagar, A. K.*	376
SOME THOUGHTS OF A PHYLOGENETIC ANATOMIST ON THE EVOLUTION OF THE FLOWER I.	*Moseley, M. F.*	394
STUDIES ON THE FLORAL ANATOMY, EVOLUTION OF THE GYNOECIUM AND RELATIONSHIPS OF THE FAMILY LOGANIACEAE.	*Tiagi, Y. D.* *Shashikala Kshetrapal*	408
THE GENERIC STATUS OF THE SOUTHERN HEMISPHERE GENTIANS.	*Philipson, W. R.*	417

SILVA AD SILENOLOGIAM 4. *SILENE TURBINATIGEMMA* ET *S. PURII* BOCQUET ET SAXENA, SPEC. NOVAE CARYOPHYLLACEARUM.	*Bocquet, G.* *Saxena, N. P.*	**423**
MORPHOGENESIS IN THE COMING DECADES.	*Maheshwari, S. C.*	**429**

Adv. Pl. Morph. 1972: 1–2

PLANT ANATOMY IN RETROSPECT AND PROSPECT

BY C. W. WARDLAW

6, Robins Close, Bramhall, Stockport, Cheshire, SK7 2PF. U. K.

This short paper, in honour of Professor V. Puri, not only pays tribute to the work of a distinguished morphologist and anatomist, but also, in a timely fashion, enables one to reflect on a very basic and therefore important branch of botanical science, i.e. plant anatomy, even though there are some today who would be reluctant to subscribe to this view. Be that as it may, it is edifying to recall that, in 1971, it is exactly three hundred years since Nehemiah Grew and Marcello Malpighi published their initial treatises on plant anatomy. (True, Robert Hooke had published his *Micrographia* in 1665 and must be accorded certain priorities; but the really systematic, initial scientific work was done by Grew and Malpighi). At the hands of the last two investigators, plant anatomy made a truly astonishing beginning; witness their numerous remarkable illustrations.

The past three centuries have seen many developments in plant anatomy—new techniques, approaches, attitudes and modes of interpretation—of which the scholars and students of today are the heritors. In a sense, each of the several phases—descriptive, developmental, comparative, organographic, physiological and causal investigations—are still with us today. There is still, as it seems, so much to be done, so much to be reinvestigated towards a better understanding of plant structure, and always so much that is new. It has been said before: if you engage on any *intensive* investigation of plants or their organs, you inevitably impinge on plant anatomy. Even biochemists who contribute to molecular biology are finding that they have to learn, or relearn, their plant anatomy.

With the passing years, one may recognise that the young—and sometimes the less young—are reluctant to spend too much time on what an earlier generation would have regarded as essential foundational studies; and this is true whether one is concerned with chemistry, medicine or botany. In an age of highly progressive technology, so many new and exciting scientific discoveries are being made daily that to some it seems little short of stupidity, or at least evidence of dullness, to spend any time on such a seemingly static subject as plant anatomy. The subject is indeed far from static but one may frankly recognize that that is its 'image' to many contemporary investigators.

So much for the retrospect and for some contemporary views. What of the prospect ?

Evidently, one may approach this query in several different ways. One could, for example, reiterate the importance of plant anatomy in any serious and deeply probing investigation of plant growth. But the fact that it is a *sine qua non* will not necessarily make it appeal to modern neophytes. It will have to be seen as an essential, dynamic component of some new and exciting field of research. This, for a preference, should deal with a major topic in botany, one assuring sustained interest. What, then, are some of the major themes for botanists of the future, which will include plant anatomists among the essential contributors ? At least three major topics, on which so far we have done no more than skirmish round the fringes, may be indicated. They are : (1) whole plant physiology, which is well nigh inconceivable without supporting anatomical studies; (2) the process(es) of differentiation, on which we have a long way to go and in which the primary evidence, often the primary clues, and the end resultant effects, all involve anatomy; and (3) specific and general organization in plants and the factors involved therein, both the evidence of organization, and the manner of becoming an organized entity, involving close attention to the anatomical aspects.

Meanwhile, plant anatomists who have taken to electron microscopy are earning big rewards and obtaining new information of great importance and fascinating interest. As in anatomy, their studies have included investigations of a descriptive, developmental and organographic (i. e. relating to form and function) nature; and studies of causality in the patterns of ultra-structures may be expected to follow at the hands of bio-physicists.

It can probably be said of plant anatomists, with some justice, that they have seldom pushed their investigations far enough. As already noted, anatomists are now making excellent use of the new highways opened up by the availability of electron microscopes. Along rather different lines, the combination of anatomy and cytology, as in the observation of high ploidies, in the development of tracheids and of some other specially differentiated cells, is producing new information of an unusually provocative and thought-inducing character. To the present writer, at least, the moral seems clear. Dedicated plant anatomists should stick to their chosen field, but they should do so with a new sense of increasing purpose in view of the essential nature of their contribution to botanical science. From time to time, botanists engage in entertaining discussions along the lines of: Is botany, or anatomy, or some other seemingly played out or static branch, DEAD ? In a gentlemanly fashion, the more urbane controversialists reach agreement that the branch in question is not dead but only somewhat dormant ! The writer has given good reasons for the view that not only is anatomy not dead but that no one can kill it. The vigour of the impact which it makes on other botanists will, however, depend on the liveliness and breadth imported into it by its exponents.

Adv. Pl. Morph. 1972: 3–27

TAXONOMIC AFFINITIES BETWEEN PIPERALES AND POLYCARPICAE AND THEIR IMPLICATIONS IN INTERPRETATIVE FLORAL MORPHOLOGY

By A. D. J. Meeuse

Hugo de Vries-Laboratorium, Plantage Middenlaan, Amsterdam, Netherlands

ABSTRACT

Survey of relevant data from various sources indicate that Piperales show a low degree of morphological advancement. It is concluded that the 'flowers' of primitive taxa represent *single* modified units and those of more advanced ones *associated groups* of such units. Divergent evolution among the oldest Angiosperms leading to an early diversification is suggested.

INTRODUCTION

The Order Piperales, generally supposed to comprise the families Piperaceae (including Peperomiaceae), Chloranthaceae and Saururaceae, and possibly also Lactoridaceae, is considered to be related to the ranalean dicotyledons by nearly all contemporary phanerogamists. The older point of view, viz., the assignment to the Piperales of a place in an apetalous or monochlamydeous taxonomic assembly (near Urticales, Amentiferae, etc.), as in the original Englerian system, nowadays has very few adherents. On the other hand, certain characteristics of the piperalean assembly, in the first place the floral morphology, are often supposed to indicate a more or less isolated position which is described in various ways, e.g., by the statement that the order represents an "early offshoot of the ranalean ancestral stock which retained some primitive characters, but also underwent some reduction, mainly in the floral region, whilst showing some advanced characters."* This phrasing, at any rate, expresses the essentials of the opinion held by systematists such as Takhtajan (1959, 1969), Cronquist (1968), Thorne (1968) and Hutchinson (1969) to mention only a few. There is a difficulty caused by the seemingly queer and, in terms of the classical morphology, almost controversial mixture of more or less ancient characteristics (such as non-tricolpate pollen and the presence of perisperm) and highly advanced ones (particularly the supposedly 'pseudo-monomerous' ovary of Piperaceae and Chloranthaceae: compare Eames, 1961). Situations of this kind, in which 'primitive' and 'advanced' characters occur in the same taxon, are explained by such authors as Takhtajan by unequal rates of evolutionary progress or heterobathmy, but, in my opinion. in this particular case under discussion, there is considerable doubt whether the Piperales indeed exhibit any highly advanced features. Several morphologists, such as Hagerup and Emberger (see Meeuse, 1964, 1965, 1966, for a discussion and references), have expressed the opinion that the floral region, more particulary the gynoecial structures, of the Piperaceae and Chloranthaceae need not have advanced very much in respect of the reproductive organs of the higher cycadopsid gymnosperms such as the Chlamydospermae. The present author believes that, at least as far as the morphology of the reproductive region is concerned, the Piperales are not so far advanced as those Polycarpicae which are in the conventional morphology and taxonomy supposed to possess the most primitive type of androecium and gynoecium (viz., the Magnoliales). Even Zimmerman (1959), whose views concerning interpretative morphology are rather conservative, is not so sure about their status (op. cit., p. 613); '*So ist meiner Ansicht nach..............für......Piperales, kaum mit Sicherheit zu entscheiden, ob hier die Apetalie primärer der sekundärer Natur ist*".

---

* Compare Melchior in Engler-Melchior (1964), p. 1948: "*Jetzt wird sie* (=*die Reihe Piperales*) *fast allgemein als stark abgeleitet betrachtet, die sich als End- oder Seitenzweig aus dem* Ranales-*Grundstock entwickelt hat.*"

Since in the classical floral morphology —which dictates the choice of the 'most primitive' recent angiospermous taxon as the group possessing the supposedly 'most primitive', i.e., monoclinous and polypalous, ranalean flower type with numerous 'petal-like' stamens and several apocarpous, follicle-like pistils — apetaly and pistillar pseudomonomery are decidedly 'progressed' characters, there can be no question but what the Piperales are in several respects in a taxonomic key position if one tries to apply neomorphological tenets in an alternative interpretation of floral morphology. Certain fundamental phylogenetic and phytomorphological issues pivot upon this controversy and thus have a considerable bearing on the taxonomic relations of the groups concerned.

A CONSIDERATION OF CERTAIN CRUCIAL CHARACTERS OF THE PIPERALES

If some of the floral characters constituting the most controversial and debatable issues, are disregarded for the time being, other characters may give us a lead.

Habit form.—The growth forms prevailing among pre- and proto-angiosperms include herbs with persistent, subterranean tubers, suffruticose and erect or climbing shrubs rather than tall trees (MEEUSE, 1967). It is exactly these habit forms that are found in the Piperales and this does not plead against a primitive evolutionary status of this taxonomic group. Similar habit forms are not at all rare in the large ranalean assembly (compare, e.g., Lardizabalaceae, Ranunculaceae, Menispermaceae, Illiciaceae, Aristolochiaceae, *Podophyllum* and other Berberidaceae and, therefore, the argument is not conclusive, but at any rate it does not rule out a possible taxonomic relationship between Piperales and Polycarpicae.

Vegetative morphology and anatomy.—The summary in Metcalfe and Chalk (1950) provides some interesting details which may be interpreted in more than one way, but are, to my mind, compatible with a primitive status of the Order Piperales.

The structure of the primary stem stele is rather unsual in that sometimes 'scattered' vascular bundles occur, a condition reminiscent of monocotyledonous stems, especially of the Dioscoreaceae (which are said to resemble the dicots in their primary stem anatomy), but ultimately a more or less continuous secondary xylem body is produced by means of a vascular cambium forming secondary phloem centrifugally and secondary xylem centripetally.

The taxomomic postition of the Dioscoreaceae may be close to the common stock of the Monocots (at least of the Liliiflorae) and some (principally ranalean) dicot groups including the Piperales (Huber, 1969). Plants with secondary growth and more or less scattered vascular bundles in the primary stem may have been of rather common occurrence among gymnospermous cycadeoid groups including angiosperm progenitors (compare the anatomical features of Dioscoreales, Aristolochiales, Piperales, *Gnetum*, and perhaps also dracaenoid Monocotyledons and Pentoxylales: Meeuse, 1961a). The growth habits of these plants include climbers and ramblers (lianas), plants with tuberous subterranean stems or rootstocks producing slender, erect or climbing shoots, suffructicose, and soft shrubs to small trees. There is, accordingly, no cogent reason to consider the gross stem anatomy of the Piperales to be 'derived' on either phylogenetic or ecological grounds.

The characters of the secondary xylem are, likewise, not per se indicative of a high degree of advancement. The chloranthaceous genus *Sarcandra* is, for instance, one of the primarily vesselless dicotyledons. Although in the Piperales the vessel perforations are apparently simple in most cases, scalariform ones have also been reported and this does neither preclude a fairly primitive condition nor a taxonomic relationship with Polycarpicae.

The number of vascular bundles in the leaf traces is usually high, crescents to almost closed circles of 3 to (usually) 5-7 or even more strands being present in the basal portion of the petiole. The number of strands in the leaf trace has been the subject of many taxonomic and phylogenetic speculations, but it is an established fact that in many of the higher cycadopsid gymnosperms (such as Cycadales, Cycadeoidales and Pentoxylales) numerous strands enter the leaf-base and it seems illogical to assume, as is so often done, that the occurrence of three- or two-strand leaf-traces is the most primitive condition in the dicots. Ozenda (1949) is of the opinion that in several more or less clearly basic, dicotyledonous groups (Magnoliales, Dilleniales) the nodal anatomy is more primitive according as the number of trace-strands is higher (5–17) and that the presence of smaller numbers (3, 2, or 1) is often indicative of a derived condition.

I believe that the nodal anatomy of the *Piperales* with a multitrace leaf-gap may, likewise, be of an ancient type. The condition in the petiole of *Chloranthus*, viz., the presence of a single deeply crescent-shaped and somewhat interrupted body of vascular tissue, suggests the lateral fusion of traces into one, the number of interruptions corresponding with the original number of individual trace bundles. The phylogenetic reduction of multitrace leaf-gaps to a three-, two- or single-trace type may, therefore, have come about not always by an oligomerisation (a reduction in number) of the strands, but sometimes perhaps by their secondary, lateral fusion.

Palynology (*pollen morphology*).—The

Piperales, although somewhat eurypalynous in the sense of Erdtman (1952, 1969), do not have the tricolpate or -colporate pollen type so common in may dicotyledonous groups. The monosulcate (anacolpate) type of pollen grain, occurring in Piperaceae, Saururaceae and some Chloranthaceae (e.g. in *Ascarina*), is undoubtedly primitive (viz., gymnospermous—cycadopsid or 'bennettitalean') and links the Piperales with the magnoliaceous Polycarpicae (the only other dicotyledonous assembly in which this ancient type is found). The aperturate and similar (polycolpoidate) pollen types are of special interest, compare, for instance, Kuprianova (1967a, b, 1969); Muller (1970). Erdtman (1969, p. 55), after having described the pollen grains of *Chloranthus* as a type with indistinct, irregular and zonally arranged aperture-like streaks, states that: "The possible occurrence in the family (Chloranthaceae) of transitions from the pollen morphology of the monocotyledon-type pollen to more dicotyledon-type habit should be investigated further". If one reads 'cycadeoid-bennettitalean type' for: 'monocotyledon-like type', a presumably more accurate idea concerning the phylogenetic relations between protangiosperms, monocots, Piperales and Polycarpicae can be obtained (see also Meeuse, 1965); at any rate the pollen characters of the Piperales as a whole are indicative of a primitive level of evolution. The pollen grains of the Piperales are binucleate when shed (Davis, 1966, and others) and this condition is clearly more primitive than the trinucleate one, at least in taxa that are not distinctly aquatic (Brewbaker, 1967).

Embryological and seed characters.— The occurrence of the so-called Peperomia-type of embryo sac (compare Davis, 1966) may be interpreted as a primitive character, because it is somewhat reminiscent of the multinucleate condition of the embryo sac in *Gnetum*. Other features (solid integuments, nearly always crassinucellate condition) do not point to a great deal of advancement either. In how far the occurrence of atropous (orthotropous) ovules in the Piperales (*Lactoris* perhaps excepted) is a primitive gymnospermous-gnetalean feature is hard to say, but it would be consistent with other evidence: the pollen characters, the small and at the time of shedding of the seed still immature (in Chloranthaceae even hardly differentiated) embryo, the presence of perisperm, etc.

Considerable emphasis has been laid on the occurrence of perisperm in Piperaceae, Chloranthaceae and Saururaceae. This is considered to be a retained primitive (gymnospermous) character, and presumably rightly so, but this has certain consequences that must be faced. If we agree that the bitegmic ovules of at least *Piper* sp. and the Chloranthaceae are still primitive, the question arises whether they should not be arillate. Large, crassinucellate and arillate ovules are rather generally supposed to be phylogenetically older than smaller, tenuinucellate and exarillate types (if only for the fact that students of seed dispersal and several ecologically minded phylogeneticists consider zoochorous, arillate seeds to be more primitive than exarillate ones— the aril being a true aril in this case and not some other arilloid structure with the same function in dispersal). Since a true aril is present in many primitive dicots, one may expect its occurrence in other primitive taxa such as the Piperales. This immediately evokes the very moot point of gynoecial homology and interpretative morphology, because a not illogical conclusion would be to seek the aril in the fleshy layer surrounding the ovule, which happens to be the pistil wall (compare Meeuse, 1964, in which paper also the vascular anatomy and the attachment of the ovule in respect of its aril=cupule is shown to be essentially similar to that of a pseudo-monomerous ovary derived from 'carpels'). This proposal, partly based on the analogy of the condition in *Gnetum* and other gymnosperms, is at variance with the conventional ideals of the Old Morphology concerning the nature of all angiospermous pistils (based on the concept of the appendicular carpel; for a discussion see also Meeuse 1965, 1966).

We shall have to return to this point later on, but it must be made quite clear once more that the traditional interpretation stands or falls with the acceptance of the flower of a 'standardised' ranalean (*Magnolia*) type as a primitive and monaxial structure. Any convincing piece of evidence that can cast some reasonable doubt on this, according to many authorities unassailable, assumption would render alternative interpretations better acceptable.

Phytochemical data.—The chemical constituents of the Piperales (Hegnauer, 1962—1969) are not indicative of a relative phylogenetic advancement of their biosynthetic mechanisms in respect of those of the Polycarpicae. The lack of some characteristics of the ranalean assembly, notably the absence of of benzyl-isoquinoline alkaloids, phytochemically distinguish the piperalean taxa from the bulk of the Polycarpicae. This could be expressed by saying that the Polycarpicae 'acquired' this very specific group of bioconstituents after the immediate progenitors of the Piperales had already become a distinct, individual group. This is consistent with the ideas expressed by, e.g., the present author (Meeuse, 1970b, Fig. 1) that Piperales originated as an early offshoot of a lineage leading to some, or all, of the Polycarpicae. The alternative supposition, viz., an 'advanced' phylogenetic status of the piperalean alliance, is not borne out by its phytochemistry; specialised families with many reduced morphological characters, such as Callitrichaceae and Hippuridaceae, usually retain 'advanced' bioconstituents (e.g., iridoid compounds in the examples mentioned, (compare Hegnauer, 1966, 1969), whereas the Piperales lack many compounds such as benzyl-isoquinoline alkaloids, polyhydroxylated acids and flavonoids, complex stereoids and saponins, etc., and do not appear to have very much advanced phytochemically.

Palaeobotanical data.—The genus *Ascarina* (Chloranthaceae), now restricted to New Zealand, New Caledonia and some neighbouring islands, most probably occurred in Europe and elsewhere during the late Cretaceous and early Tertiary (see Erdtman, 1969, p. 99). The genus must have been much more wide-spread in the past, and the pattern of distribution of certain recent taxa is accordingly of the nature of relict refugia, which conclusions are better compatible with the assumption that the Piperales are old and primitive than with the alternative idea of a considerable degree of advancement of the order. According to the review by Muller (1970), the first positive identifications of fossil pollen types can be made in Senonian deposits and among these early records is *Ascarina* of the Chloranthaceae. Muller (l.c. p. 423) agrees with Kuprianova (1967b) that sometimes irregularly monosulcate to multicolpate pollen grains of the Chloranthaceae may represent a transitional stage between the monosulcate cycadeoid (=bennettitalean) type and the dicotyiedonous type. The presence of these primitive grains in the Upper Cretaceous is not at all surprising, and their identification as partly belonging to a more or less clearly primitive surviving genus (*Ascarina*) not altogether unexpected. The fixation of the overall morphology at the generic level most probably had already been completed by that time and the Piperales as an order must not only be appreciably older than the Upper Senonian, but in their morphology must also have retained other archaic features on account of that fixation.

Conclusions.—What factual evidence there is pleads in favour of a generally primitive status of the order of the *Piperales*, and the early trends of specialisation have, as I shall attempt to demonstrate, not quite veiled the phylogenetic significance of their floral morphology. The fossil records and the present range of distribution of the Piperales indicate that the group was in the past distributed at least in all warmer and temperate regions of the world. It must have been much more 'prosperous' and its most conservative representatives could in many parts of the world have divergently and more or less simultaneously evolved into their derivatives such as the rather heterogenous Polycarpicae and perhaps Liliiflorae, etc., on a fairly broad front, so that by the end of the Cretaceous many basic groups had become fixed, and thus recognisable, in the fossil record.

This brief compilation confirms that it is only the morphology of the reproductive region which is prohibitive to proclaiming the Piperales altogether very primitive dicots in terms of the conventional floral morphology. The question always boils down to the alternative of a monaxial modified shoot adapted to reproduction (the old concept) versus a more complicated anthocormoid structure (see Meeuse, 1966 for a survey and for relevant references; Melville, 1969). In the case under discussion the so-called 'flowers' of the Piperales, often (but not in the family Saururaceae) consisting of only a unilocular one-ovuled pistil and one or a few androecial element(s) surrounded by a perigone or even completely 'naked', are either derivatives of an alleged primitive flower of the *Magnolia* type with numerous steril and fertile floral appendages and the product of excessive oligomerisation and reduction, as the classical theory has it, or portions of an anthocormoid structure which have not changed so much barring some oligomerisation (Meeuse, 1965, 1966) and not necessarily homologous with a whole ranalean flower (representing a special, monoclinous anthocorm). Does either taxon —piperalean or ranalean— provide any additional evidence. Let us examine certain peculiar characteristics of the reproductive region of some Polpcarpicae and other relevant data.

MOOT POINTS CONCERNING THE FLORAL MORPHOLOGY OF THE POLYCARPICAE

The consequences of a priori homologisations.—In the classical flower morphology the reproductive zones are supposed to consist of units called 'flowers' (or *anthostrobili*, etc). or to contain a number of such units (in inflorescences). Each unit or flower, in the primitive condition, is supposed to be ambisexual (monoclinous), monaxial and uniformly constructed, the central floral axis bearing a number of either fertile or sterile 'appendicular' units called sepals, petals, tepals or collectively perianth, perigone, calyx, and corolla when they are non-fertile (asporangiate), and stamens and carpels or, collectively, androecium and gynoecium, respectively when they are fertile (sporangiate). Other sterile elements such as staminodes, pistillodes, nectaries, disk-lobes, etc., do not concern us here. All so-called appendicular elements including the sporangiate ones are supposed to be leaf homologues of a sort, at least they are thought to represent non-axial elements with a number of leaf characteristics (Eames, 1961, Carlquist, 1970). A primitive angiospermous flower, supposed to be closely approached by the flowers of Magnoliaceae, *Eupomatia* and some other Polycarpicae, is assumed to have still 'foliaceous' anthers, numerous free monocarpellate pistils and usually a number of sterile appendages (the perianth segments). All other flowers are somehow to be 'derived' from this prototype. Doubt has been cast on several of these dicta: (a) the majority of the conventional flowers may be polyaxial, (b) there is no reason to accept the ambisexual condition as the only primitive one (dicliny is almost certainly primitive in some families: Meeuse, 1965, Chapter IX, 1966, 1970a), (c) the androecial morphology is much more complicated (see, e. g., Van Heel, 1966, 1969,) and will be discussed in the next paragraph, (d) a pistil need not always be carpellate, (e) a carpel may be a much more complex morphological structure than it is supposed to be in the classical interpretation. Starting from a different, polyaxial basic morphological unit or anthocorm (and using some or all of the alternative dicta), one may come to altogether different homology relations and encounter a far greater diversity of phylogenetic development. The morphological nature of essentially sterile (asporangiate) floral parts is so varied that even in the conventional floral morphology the heterogeneity of this group of elements is generally admitted. The controversial issues principally concern the sporangiate elements.

'Stamens' of various kinds.—The broad and flat microsporangiate floral organs of some Nymphaeales, nearly all Magnoliales, and a few minor taxa are either primitive or highly derived. That they would be 'primitive' is more a matter of wishful thinking than of factual evidence, and one would atleast expect a primitve structure to be morphologically and anatomically less complicated than the more common type of male reproductive organ, a slenderly stalked and nearly always tetrasporangiate anther. There are several ways to 'derive' the latter type of stamen from a flat, 'petal-like' sporangiate organ, at least on paper, but there is of course a prerequisite which is almost invariably but quite erroneously taken for granted, viz., the fundamental question if all tetrasporangiate pollen-producing organs traditionally called 'anthers' are indeed morphologically equivalent (homologous) in every respect, and this is exactly the rub. This is not only a matter of the alternative postulates of monaxial versus polyaxial flowers, but also of the alternative possibilites of a simple versus a more complicated androecial structure as we shall see.

One may safely state there are only two main floral theories in which the flowers are considered to be much more complicated than in the classical theory, viz., Neumayer's anthocorm theory of 1924 (based on earlier ideas such as those of, e.g., Delpino-Wettstein), modified by the present author (Meeuse, 1965, 1966) and Melville's gonophyll theory of 1961 (see Melville, 1962, 1963, 1969). The gonophyll hypothesis has two great disadvantages, viz., the very slender phylogenetic evidence, and the introduction of a morphological 'unit' (the gonophyll) which is not clearly defined as an axial, foliar or 'mixed' organ. The occurrence of ambisexual fertile organs in certain glossopteriod pteridosperms, as reported by Plumstead, is now considerd to be highly improbable (compare, e.g., Emberger, 1968, p. 440). The organs in question were apparently either micro- or mega-sporangiate (the fact that the sporocarps of the Marsileales, possibly descendants of glossopterid seed ferns, (see Meeuse, 1961b), are ambisexual, is not at all decisive, because this may have been one of the reasons for their survival and may, therefore, be exceptional). Another objection I raised before is that one must not make a 'jump' from a special, not to say rather enigmatic, kind of pteridospermous reproductive organ to an androecial (or gynoecial) element of an angiospermous flower without taking certain homology relations (megasporangium→ovule, cupule→true aril etc.) into account. Melville not only introduces a basic bisexual gonophyll, but sometimes postulates a compound 'gonophyll of gonophylls' as a fundamental archetype, although the fossil evidence for such a complex structure is altogether non-existent. Melville's studies have shown that an alternative approach to floral morphology is possible, but his interpretation of the evidence concerning the complicated nature of the reproductive region of the Flowering Plants and their early morphological divergence must be 'translated' into an interpretation in terms of complex fertile, axial organs and associated bracts, in fact, in terms of the anthocorm theory, which theory is better in accordance with paleobotanic records and with standard morphological principles.

Clarity and simplicity in terminology and in definition require the introduction of some terms (which I had previously avoided), so as to emphasise the fundamental inequality of floral elements treated as homologues in the classical theory. We need not concern ourselves with the question of ambisexual or unisexual 'flowers', as long as the postulate of the unisexual sporangial truss supported by a bract as a part of an anthocorm is accepted. The essential nature of the truss is not so much its mode of branching (which could be 'racemose' or 'fascicled' or more or less dichotomously bifurcating) as the absence of bracts supporting each separate element.

'Trusses' of stalked ovulate or microsporangiate organs have been shown to be common among Mesozoic cycadopsid groups such as Caytoniales, Pentoxylales and even Nilssoniales. Each element of a female truss consists of an ovule surrounded by its stalked cupule (=aril) or sometimes of several ovules in a common stalked cupule. Essential is that the cupule is borne on the stalk and ovules on the cupule, so that in the ultimate and hemi-angoispermous to angiospermous conditions the, mostly single, ovule is originally not terminal on the stalk (Meeuse, 1964). The male counterpart bears stalked, and ultimately nearly always tetrasporangiate synangia, which although they may have incorporated other organs (a cupule equivalent for instance) are essentially comparable to an ovule on its stalk (=funicle, because ovules and anthers are, phylogenetically speaking, ultimately homologous, but for practical purposes, alternative and in several respects at any rate topologically equivalent entities* Meeuse, 1965, 1966).

To avoid ambiguity and the possible confusion between terms used by Neumayer, Melville and perhaps others, I shall only retain the term *anthocorm sensu mini* (see Fig. 1*a*). Each bracteated side-branch, if male (microsporangiate), will be referred to as a polyandron (PA), and if female (megasporangiate), as a polygynon (PGy), and they both a polygona (PGo).

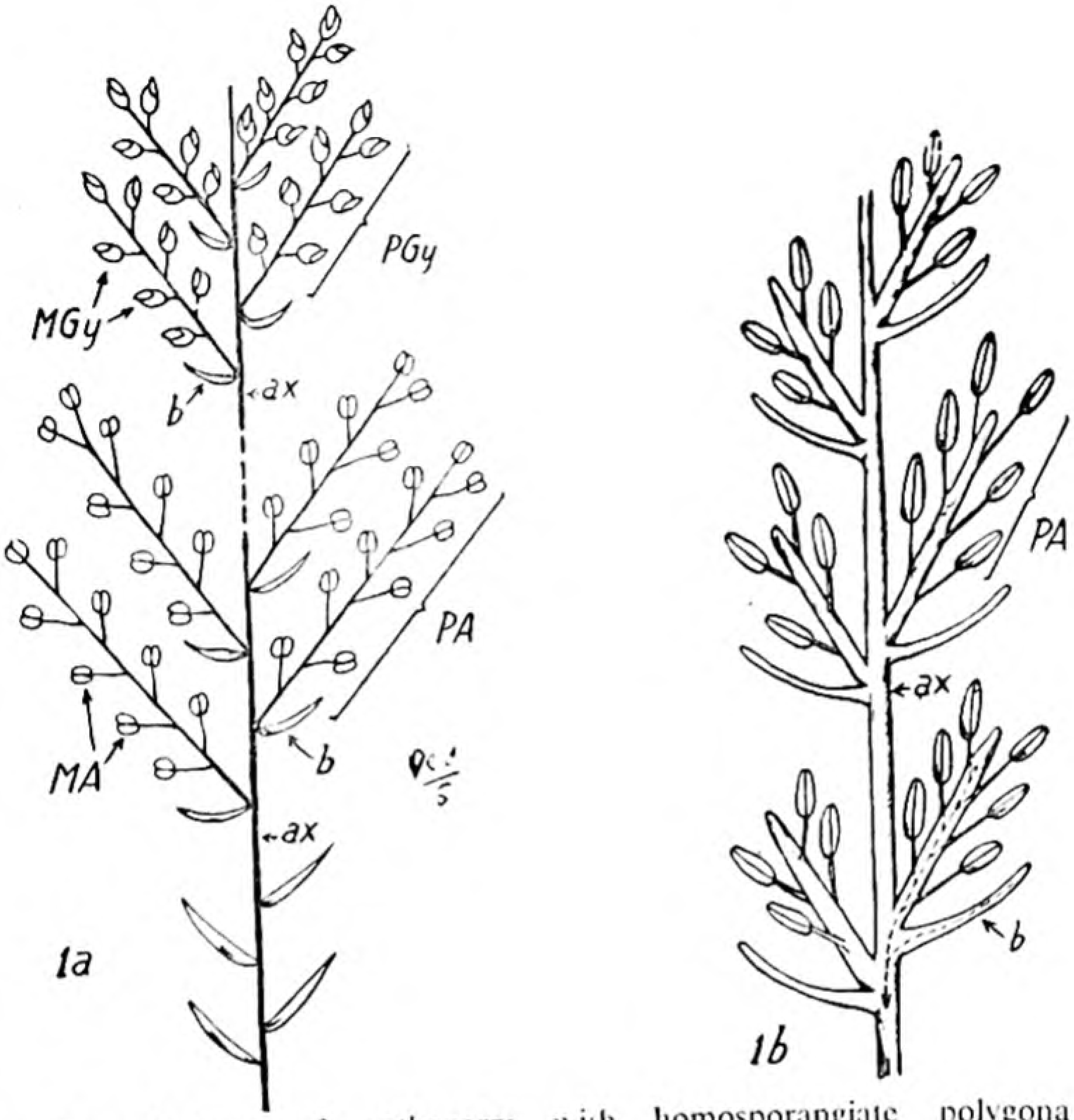


Fig. 1 a. General type of anthocorm with homosporangiate polygona (=gonoclads). b. Male anthocorm, with vasculature in one PA drawn in.

ax, anthocorm axis; *b*, bract of gonoclad = stegophyll; PA, polyandron (usually) equivalent to androclad); MA, monandron (= anther) on filament; PGy, polygynon (usually = gynoclad); MGy, monogynon (= ovuliferous cupule) on stalk (usually subsessile to sessile in advanced forms).

The subtending bracts will be called PAB(racts) and PGyB(racts), respectively. Generally speaking, a polygonon is equivalent to what I have previously called a gonoclad (and a polyandron to an androclad, a polygynon to a gynoclad) but the sporangiate structures are not always strictly monaxial and do not always bear their monogona in a helical arrangement and may be fascicled, dichotomously branched, etc. The terms gonoclad, androclad and gynoclad are sometimes used in the present paper when there is no possibility of confusion or ambiguity. The ultimate parts of a polyandron and polygynon are to be called monandron (MA) and monogynon (MGy), respectively (see Fig. 1b, in which also the association of the vascular supplies of each polygonon and its subtending bract is indicated).

---

* The opinion of Van Heel (1969, based on anatomical features, and of other workers such as Celakovsky, Guedes and Molliard), based on teratology, that ovules are equivalent to pollen sacs is not necessarily at variance with this conclusion (because this depends on my interpretation of the aril = cupule = chlamys as an initially pluri-ovulate structure) and, in actual practice, in Angiosperms the arillate ovule is usually equivalent to an anther, but sometimes, *e.g.*, in Piperales and in Winteraceae, an anther is equivalent to a pistil.

The androecium of a conventional angiospermous flower is, according to my interpretation, usually the phylogenetic derivative of all polyandra of an ancestral anthocorm (namely, in those cases when the flower is a modified anthocorm; exceptions will be dealt with later on), but what is the fate of the associated bracts? The PABs are quite often retained and represented by tepals, sepals or petals, each opposite the associated PA or what is left of it. Conceivably, they are occasionally 'lost' by complete reduction, leaving 'naked' PAs. A third possibility is that the PAB enters into a closer association with its axillary PA. The PAs may show different trends, viz., a retention of the orginal condition, each PA having several MAs, or an oligomerisation, ultimately each PA retaining only a single MA (see Fig. 2.). These suppositions are very similar to Melville's suggestions of the derivation of androecia from 'androphylls'. In a whole anthocorm changed into a flower, divergent trends must have caused as many additional, divergent evolutionary developments.

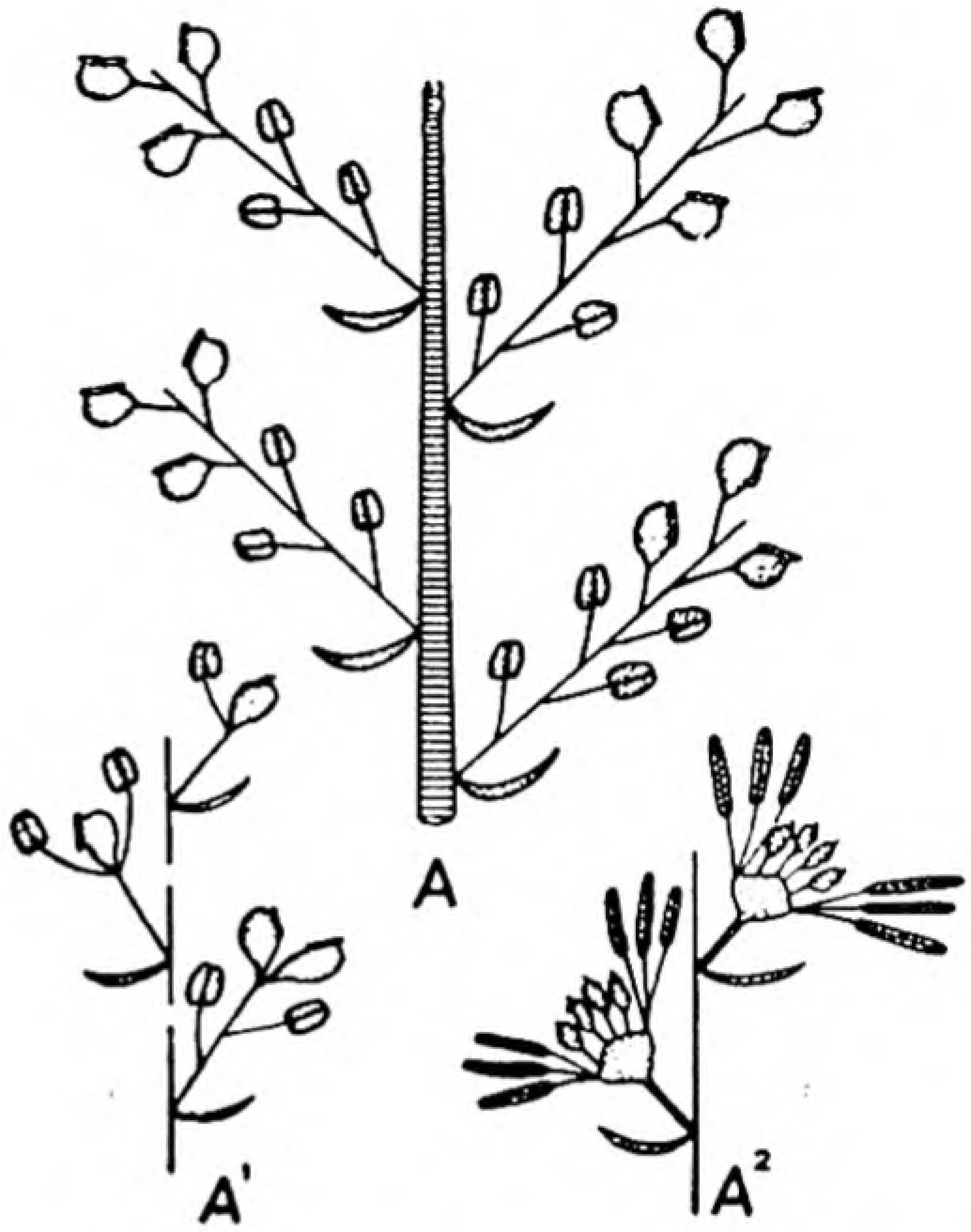


FIG. 2A-A². A. Androgynous anthocorm with four androgynoclads or polygonons (=PGo). Each PGo plus its stegophyll represent the archetype of the conventional 'flowers' of e.g., the Saururaceae if the cladic part of each PGo is foreshortened. A¹. Androgynoclads forming the conventional flowers of Piperales by a tendency towards oligomerisation of the number of MAs and MGys per PGo (cladic part of the so-called flower extended in the drawing), diagrammatic. Top: *Sarcandra* type; bottom left: Piperaceae; bottom right: Saururaceae. A². *Eupteleu*, 2 androgynoclads (conventional 'flowers'): cladic part of PGo broadened in their distal portion where MAs and MGys are inserted (semi-diagrammatic).

Presuming that the number of PA-PAB units of an anthocorm was fairly large and indeterminate and that the units were helically arranged, two principal divergent developments can be distinguished, viz., a retention of the large and indeterminate number of still helically arranged PA-PAB units (as in several Polycarpicae), or a reduction (oligomerisation) of the units to a smaller and determinate number of cyclically arranged elements (as in Clusiales, Dilleniales, Malvales, Cistales, etc.). If in the latter case, each PA—PAB unit still contains a fairly large number of MAS, the arrangement of the stamens is frequently at variance with the dicta of the classical floral theory and the conventional floral diagrams, which postulate either acyclic (=helically inserted) floral parts or alternating and usually 'isomerous' whorls of elements. There may, for instance, be 'obdiplostemony' or centrifugal stamen development. One of the conventional explanations of these 'irregularities' partly inspired by anatomical data, is the postulate of chorosis or dedoublement or splitting of primordia (this is apparently still the favoured explanation of the German morphologists and developmental anatomists, compare Mayr, 1969, also for references to similar recent studies by Eckert, Gelius, Hiepko, Leins, and others). Van Heel (1966), after extensive morphological and anatomical investigations of principally Malvales, came to a different conclusion, viz., that in this order the androecia are 'stachyosporous' and consist of usually 5 or 10 units, sometimes all or some of them anatomically associated with the usually pentamerous corolla, sometimes epipetalous. Van Heel, like Mayr and others, found that cases of polyandry (in Malvales, Myrtales, Saxifragales, etc.) ontogenitically nearly always start as protuberances of the primordial floral apex, each protuberance subsequently giving rise to a number of stamens. This apparently 'secondary' development is, in the classic interpretation, attributed to the splitting of the primordium into identical multiplets, each group of multiplets being morphologically (in the floral diagram, etc.) equivalent to a single stamen.

I cannot see why this is the only explanation, because a PA, a lateral and fundamentally axial organ, would also develop epipetalously or episepalously as a single primordium and differentiate later by developing secondary protuberances (its later appendages), etc. Either explanation has its merits, but their consequences are altogether opposed: a secondary multiplication of a single stamen supposed to be an appendicular organ would produce only appendicular organs (and this does not clash with the classical concept of the monaxial flower), whereas the other explanation implies a relation between a bract and an axillary, axis-like, PA, which fits better in the concept of the polyaxial flower. It follows that other evidence of a fully or partially axial nature of the reproductive organs (such as the anatomy of the so-called laminar stamens of *Victoria* to be discussed presently) would refute the dedoublement hypothesis.

We must first return to our starting point of the morphological nature of what is called a stamen in the classical meaning. It is clear in my opinion, that in fact two or more types may exist, because there must have been several, early divergent pathways of androecial evolution. In a primitive, 'polyandrous' androecium each microsporangiate element (stamen) is the phylogenetic derivative of only one of the MAs of a PA. If the androecium consists of a number of PAs each of which is reduced to a single MA (as in, e.g., nearly all Liliiflorae, Cyperales, Cornales and many sympetalous dicots), a stamen is to all intents and purposes also equivalent to a single PA, but it represents at the same time an (originally biaxial) MA. There is a third and comparatively rare type of androecial element which is the allegedly most primitive, petal-like type of 'stamen'. In several publications the present author, (Meeuse, 1963, 1965, 1966) following up certain suggestions by, e.g., Ozenda (1952), gave an altogether different interpretation of these peculiar reproductive organs. The explanation of their morphological nature by Melville is essentially the same, but expressed in terms of his gonophyll theory and, therefore, in his interpretation some essential aspects of the dual nature of certain types of genitalia are not sufficiently taken into account.

The starting point of my interpretation is the assumption that among the Angiosperms the phylogenetically oldest type of androecial element is a stalked and normally 4-saccate microsynangium, in other words, the M.A. The two most striking features of the flat stamens of many Magnoliaceae, Annonaceae, Nymphaeaceae, etc. are the separate bisaccate thecae (suggesting 'half' anthers) and the 'double' vascular system (Ozenda, 1952; Melville, 1963, 1969).

The supposition was made (Meeuse, 1963) that this organ is a complex structure, an amalgamation of a PA (androclad) reduced to a single MA with its PA bract (each with a separate vascular trace or set of traces), the phylogenetic fusion being of the nature of a neotenic (i.e., precocious and hence simultaneous) development. and the bilateral modification of the axial portion (the oligomerised androclad, practically a single MA). I dubbed this condition 'pseudo-phyllospory' in some previous publications, but this seems to have led to some misunderstanding. The factual evidence was until recently rather slender —the evidence was in fact mostly indirect — and a number of characteristics such as its development as an apparently single entity, seemed to refute the idea of a dual morphological nature of the alleged primitive ranalean stamen in the eyes of many contemporary phytomorphologists.

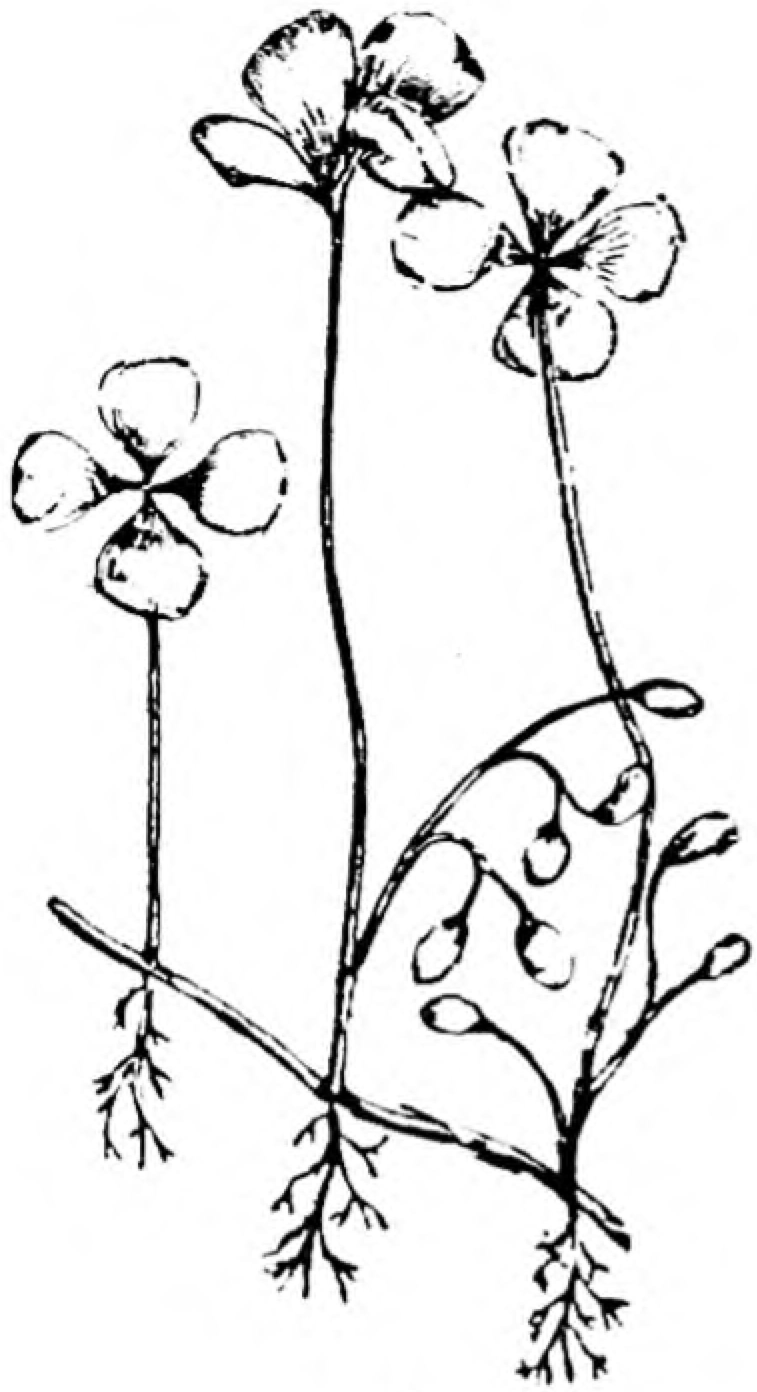
The recent rediscovery of the vascular anatomy of the 'stamens' of *Victoria amazonica* (first described in 1899 by Knoch who failed to see the scope of his discovery, however) by Heinsbroek and Van Heel (1969) contributed very cogent arguments in favour of the dual morphological nature of these strap-shaped organs. In the giant water-lily the male reproductive organs exhibit a vascular anatomy which in its details does not only confirm the presence of a 'double' vascular supply, but also indicates the presence of characteristics of a leaf-like (lateral or appendicular) kind of anatomy in one of the two trace systems, *viz.*, in the one on the abaxial side and of an axial one with inverted auxiliary bundles on the adaxial side. Heinsbroek et al. compare this structural detail with those of the ovuliferous scale of the Coniferae and there is, accordingly, every reason to apply the same interpretation to all corresponding laminose male structures of other Nymphaeales and of the Magnoliaceae. Melville explains this organ as a modified androphyll, assuming a primary dichotomy in a sporangial truss as the reason why there are two separate bisaccate thecae, but this is, among other things, at variance with the much more common occurrence of bithecate (=tetrasporangiate) male organs with a single vasucular strand in the filament (the MAs) . The morphology and ontogeny of the organ of dual phylogenetic origin may be compared with that of an ovuliferous cone scale of a Conifer as we have seen, and its development as if it is a single organ does, therefore, not rule out a phylogenetic adnation and ultimately utter amalgamation of two organs of an entirely different nature into a seemingly single entity combining and 'mixing', in a rather singular way, the characteristics of both original constituents (for an exhaustive discussion of this process of congenital fusion the reader in referred to Van Heel, 1969).

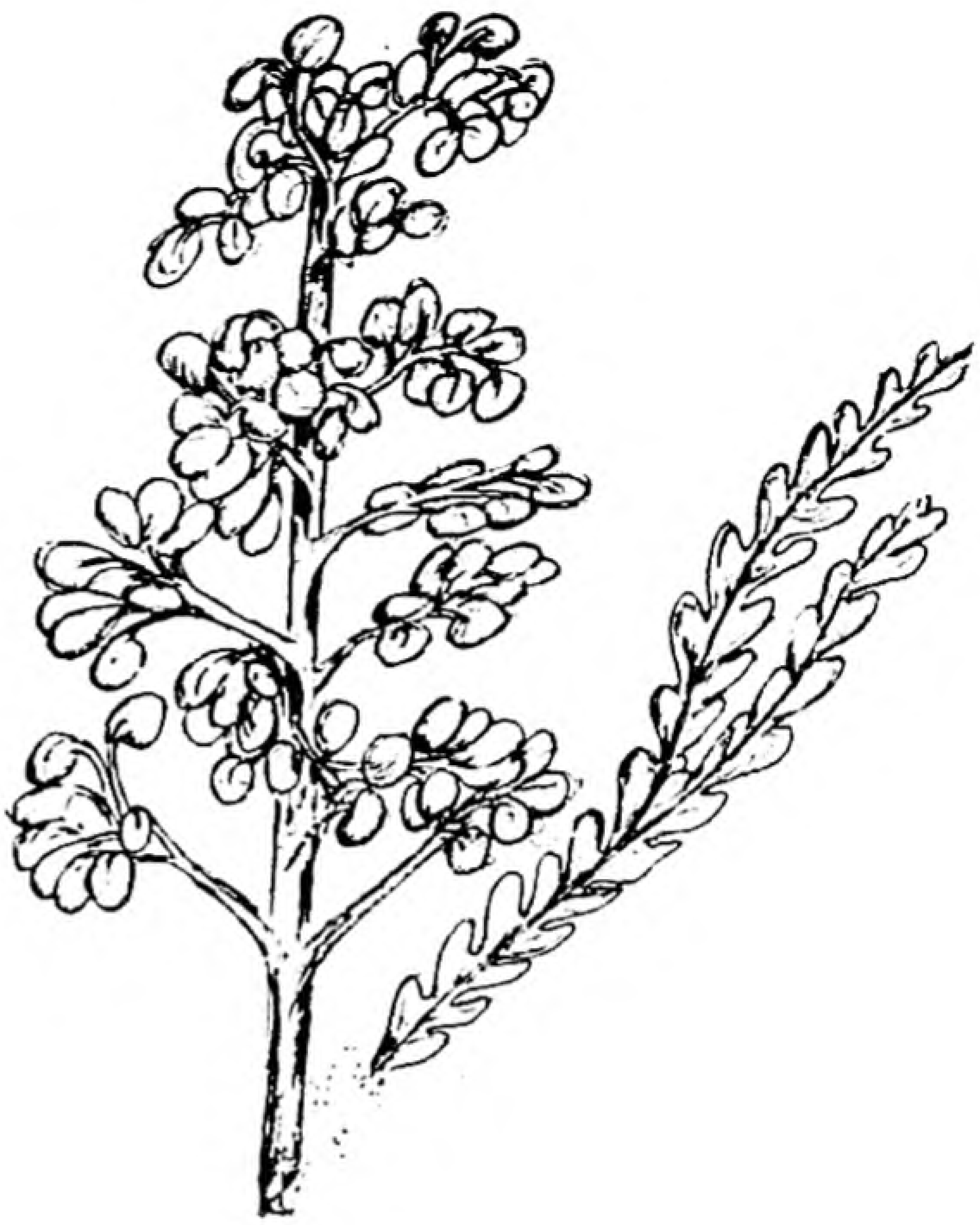
Some consequences of the new interpretation.—It follows that microsporangiate, flat and broad organs bearing a lateral or nearly lateral bisaccate theca on each flank, are complicated and derived structures instead of primitive 'archetypes' of all other conceivable types of 'stamens'. This conclusion is of fundamental importance in interpretative floral morphology and in phylogenetic taxonomy: the so-called primitive 'stamen' of several ranalean groups (for which I here propose the name of monandronoid) is advanced in respect of the monandron, hence the magnolioid type of flower can not be the most primitive one and the Magnoliales are not the most primitive living Angiosperms in the sense that the floral region of the pre- and protoangiospermous groups must have looked like those of the recent magnoliaceous taxa.

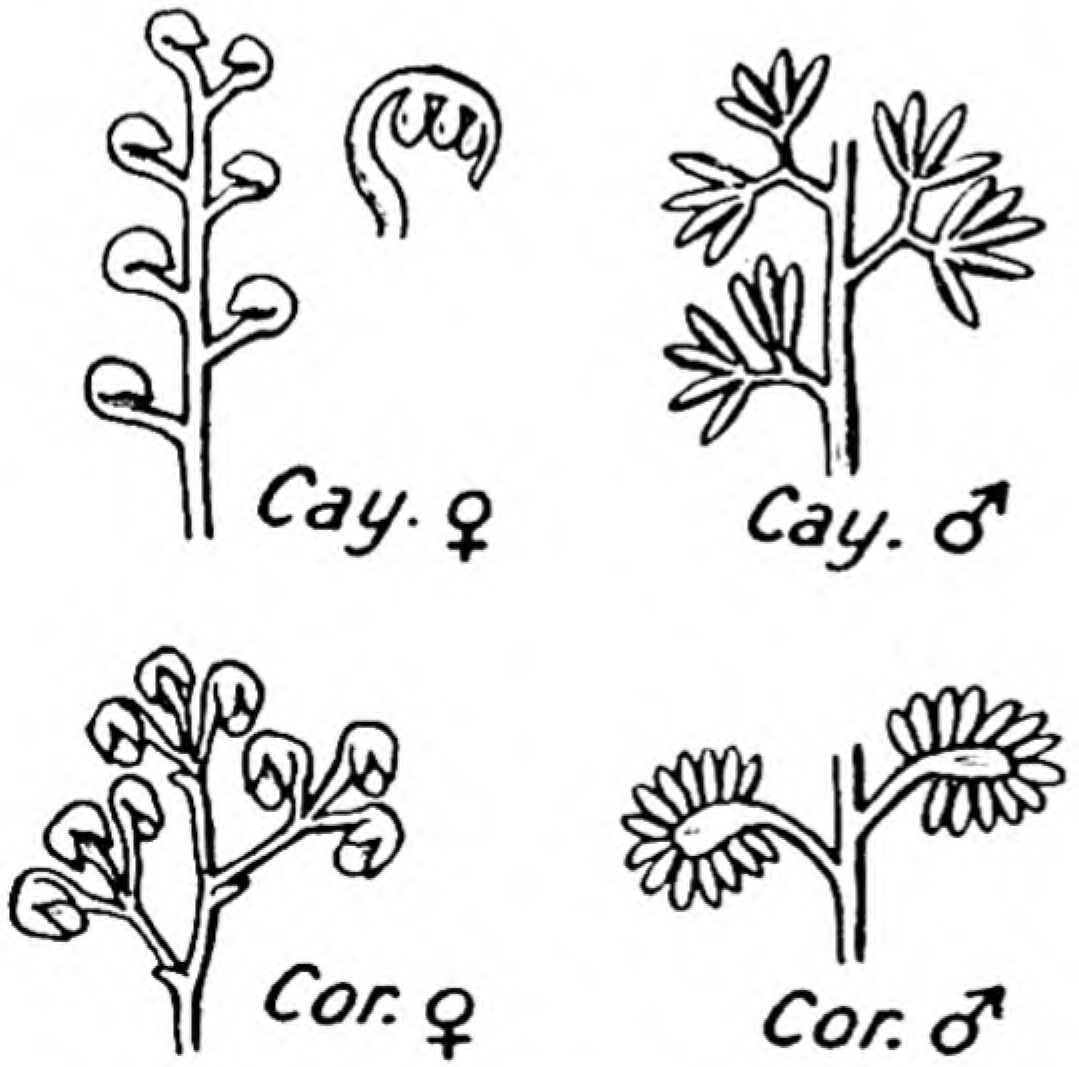
The far-reaching consequences of this interpretation must be faced, in the first place its bearing on the probable morphological nature of megasporangiate floral elements. There is every reason to assume that the peculiar monandronoid has its female counterpart and that organs which are topologically, and also in other respects, the equivalent of a monandroid must also be the product of a phylogenetic and morphogenetic amalgamation of a sporangiate axis-like structure (PGy, or gynoclad) with an associated lateral organ (the subtending PGyB), to form a polygynonoid entity, a phyllodic complex organ. However, this need not compel us to interpret all pistillar structures and gynoecia as assemblies of laminar polygynonoids, and we should rather keep an open mind as regards the possible occurrence of pistils equivalent to gynoclads (PGys) or to a single MGy. After all, the ovuliferous truss (gynoclad or polygynon) is phylogenetically older than the polygononoid to whose origin it contributed, so that such an ancient type of gynoecial element may well have been retained in some primitive group or groups of the Angiosperms. The interpretation of the magnoliaceous stamen as a monandroid is not only one and an alternative one, involving the so-called cortical vascular system of the floral axis must be seriously considered (Meeuse, in preparation).

Cupulate ovules, polygynons, carpels and pistils.—The classical interpretation of gynoecial structures reduces every pistil to a 'basic' constituting entity, an alleged appendicular organ called the carpel. This concept requires a number of additional postulates to account for all characteristics of pistillar strucutres that are not easily conformable to that simple interpretation (such as the concept of the peltate carpel with a Querzone, or of a 'diplophyllous' organ etc.). An ample discussion can be found in Moeliono (1966, 1970), who showed that the postulation of an axial nature of the placental regions is an equally acceptable, alternative hypothesis and that it provides a better explanation of the gynoecial morphology, anatomy and ontogeny of, e.g., Centrospermae (sensu lato) and Primulaceae.

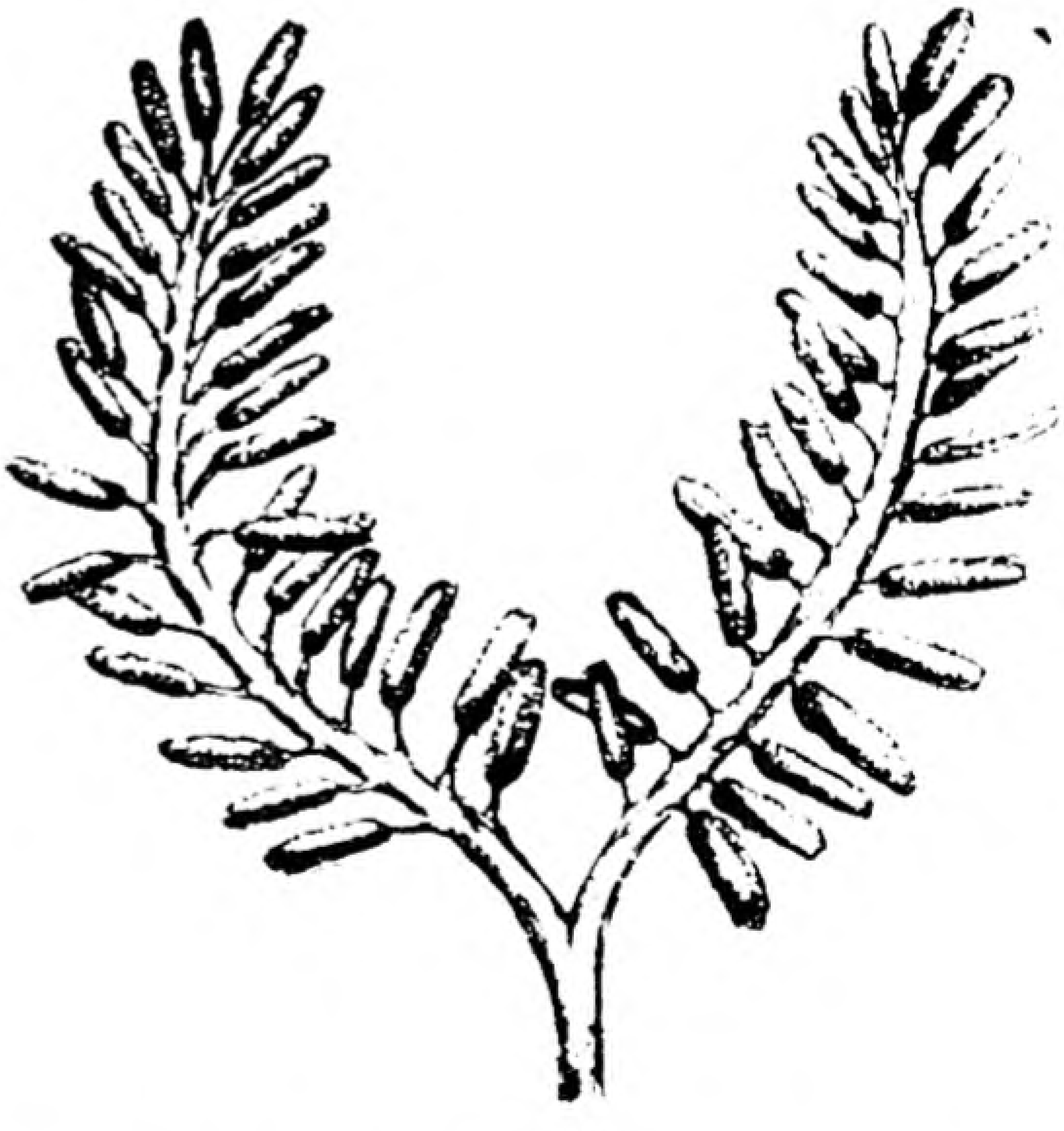
The indentification of the megasporangiate organs with subordinate elements of an anthocorm (gynoclads, etc.) does not only provide a common prototype for all gynoecial structures, but also enables the topological comparison with the microsporangiate constituents of the same anthocorm. A truss of ovule-bearing stalked cupules subtended by a bract is the basic polygonon—PGyB unit. Each cupule is usually one-ovuled in the higher Cycadopsida, pluri-ovulate, however, in Saururaceae, Lactoridaceae and I think, Winteraceae and some other ranalean taxa) and, for practical purposes one can say that barring some exceptions a polygynon is a truss of chlamydote arillate ovules. Such a truss is represented by the placental region in the more advanced


3


4



5


6

FIGS. 3-6 Fig. 3. Proto-anthocorm in *Marsiliales*: trusses of 'sporocarps' on a common cladic organ associated with the stalk of the frond. Fig. 4. Proto-anthocorm of *Hydropteridangium marsilioides*. Fig. 5. Anthocorms (or portions of anthocorms) of *Caytoniales* (Cay.), and *Corystospermaceae* (Cor.) female and male, respectively. Fig. 6. *Zuberia zuberi* (male): bifurcate male, anthocormoid fertile structure.

ovarial structures and has a close association with its PGyB. In the past a great deal of mental energy has been spent in efforts to 'derive' the various types of ovaries from a common prototype, viz., the apocarpous ranalean gynoecium (i.e., an assembly of ovuliferous follicles with ventral-marginal placentation), by oligomerisation, fusion, concrescence and reduction, etc. The various types of placentation were, likewise, explained as secondary modifications of an originally marginal position of the placental regions. The painstaking studies by Moeliono have shown that other types of placentation, more particularly the 'free central' types of Centrospermae and Primulaceae, need not have arisen from a condition as found in the Polycarpicae with follicular pistils. There is also no reason to assume that a pluricarpellate and unilocular ovary with laminal placentation (as in Nymphaeaceae s.s., etc.) is a derivate from an apocarpous group of follicular pistils that underwent a whole series of changes: flat or in transverse section crescent-shaped polygynonoids with marginally or laminally borne ovules may have joined laterally to produce 'at once' a 'syncarpous' pistil (Meeuse, 1966).

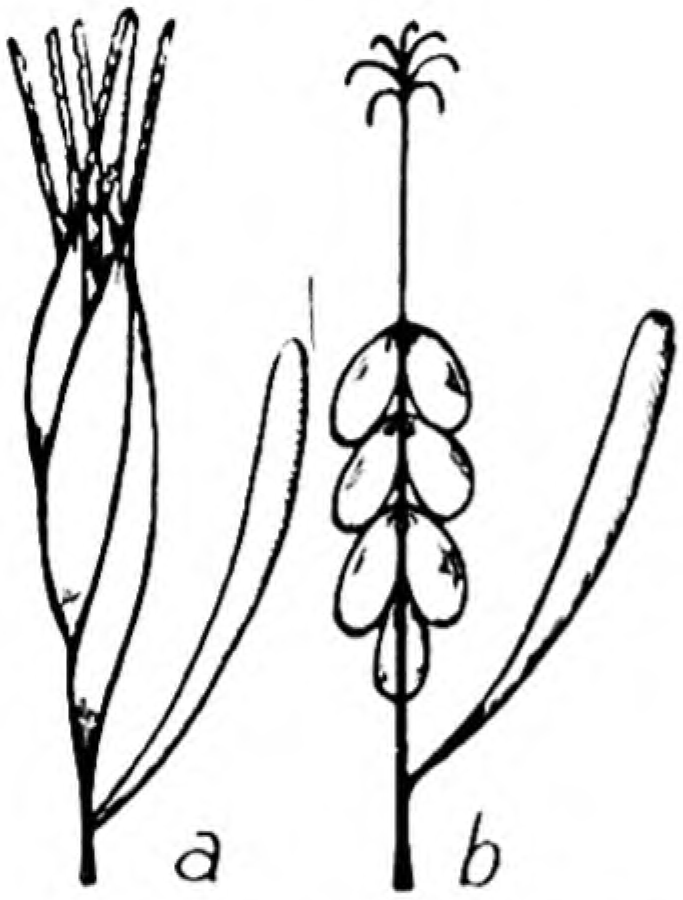


Fig. 7. So-called 'flowers' of certain Centrolepidaceae, most probably PGys=gynoclads with the MGys associated into a phalanx; at left: each MGy with its own stigmatic portion, at right: MGys (and cladic part of PGy?) have formed a common style.

The question arises whether there may be any cogent evidence comparable to the telltale anatomical structure of the monandronoid of *Victoria*. I shall return to this question presently. As is frequently the case in the androecium, the ovuliferous PGys of a primitive anthocorm may have retained their morphological structure in the floral region of certain angiospermous taxa. Considering that the primitive ovules are surrounded by a cupule derivative developing into a true seed aril, we may assume that primitive taxa reputed to possess exarillate seeds and pseudomonomerous one-ovuled ovaries may never have attained the phylogenetic level of the polygononoid carpel and retained primitive polygynons (=gynoclads) bearing several monogynons (=arillate ovules on a stalk=funicle) or PGys reduced to a single MGy. This condition may be present in some taxa of the Piperales, in the majority of the Laurales, in Cyperaceae, Pandanales and in a few other groups.

As I have pointed out some years ago (Meeuse, 1964), the vascularisation pattern of one-ovuled pistils does not enable us to discern between a one-ovuled derivate of a ranalean polygynonoid (or of a 'syncarpous' assembly of such complex organs) and a derivate of an ovuliferous cycadopsid cupule. Another approach viz., a comparative study of male and female structures of the same or of closely related species and of conditions in the Chlamydospermae and in certain angiospermous taxa, is therefore indicated. The study of the morphology of the reproductive organs of the gnetalean gymnosperms and of the Piperales (and some other angiospermous groups) is hampered by the rather excessive oligomerisation of certain elements and by a complication associated with the sex distribution. As I have pointed out (Meeuse, 1965, Chapter IX), the 'bisexuality' of the floral region of many angiospermous groups is a secondary condition derived from the original dicliny of their gymnospermous progenitors rather than a primitive one, transitional stages with (bisexual) polygonons (androgynoclads) occurring in the Chlamydosperms and presumably still in a few groups of primitive angiosperms. A reconsideration of the floral morphology of certain diclinous to polygamous Dicots, such as Monimiaceae and Cercidiphyllaceae and of the Eupteleaceae may, therefore, be elucidating.

PRIMITIVE DICOTYLEDONS WITH ANCIENT ANTHOCORMOID FLORAL REGIONS

Difficulties, inconsistencies or even controversies met with in interpretations, by means of classical principles, of the floral morphology of certain genera or families clearly stress the untenability of some of the conventional tenets. The staminate structures of *Cercidiphyllum*, consisting of a bract subtending a cluster of 8-13 stamens, are helically arranged on the male reproductive axis, and the groups of 2-6 coaxial 'pistils', each of which is likewise subtended by a membranous stegophyll (see Fig. 8), are also acyclic (Swamy and Bailey, 1949). Several workers cited by Swamy et al. have stated that the acyclic arrangement and the abaxial orientation of the ventral sutures of the 'carpels' form a serious, if not an insuperable, obstacle to interpreting the female reproductive structure (i.e., a coaxial group of 'pistils' or 'carpels') as a single flower. Anatomical features do not provide the solution and the only way out was to draw the conclusion that each pistil represents the vestige of a much reduced (and subsequently in its gynoecial parts extremely oligomerised), ultimately 'naked' flower. The male unit (a cluster of stamens) would represent the counterpart, the remains of a single (male) flower. As I pointed out in my discussion of the status of the Piperales, the postulation of such extreme floral advancement in a taxon that is in many other respects undoubtedly primitive (compare e.g., Lemesle, 1946, 1955) and known in the form of clearly identifiable fossils since the Cenomanian, is carrying the idea of heterobathmic evolution beyond the limits of credulity.

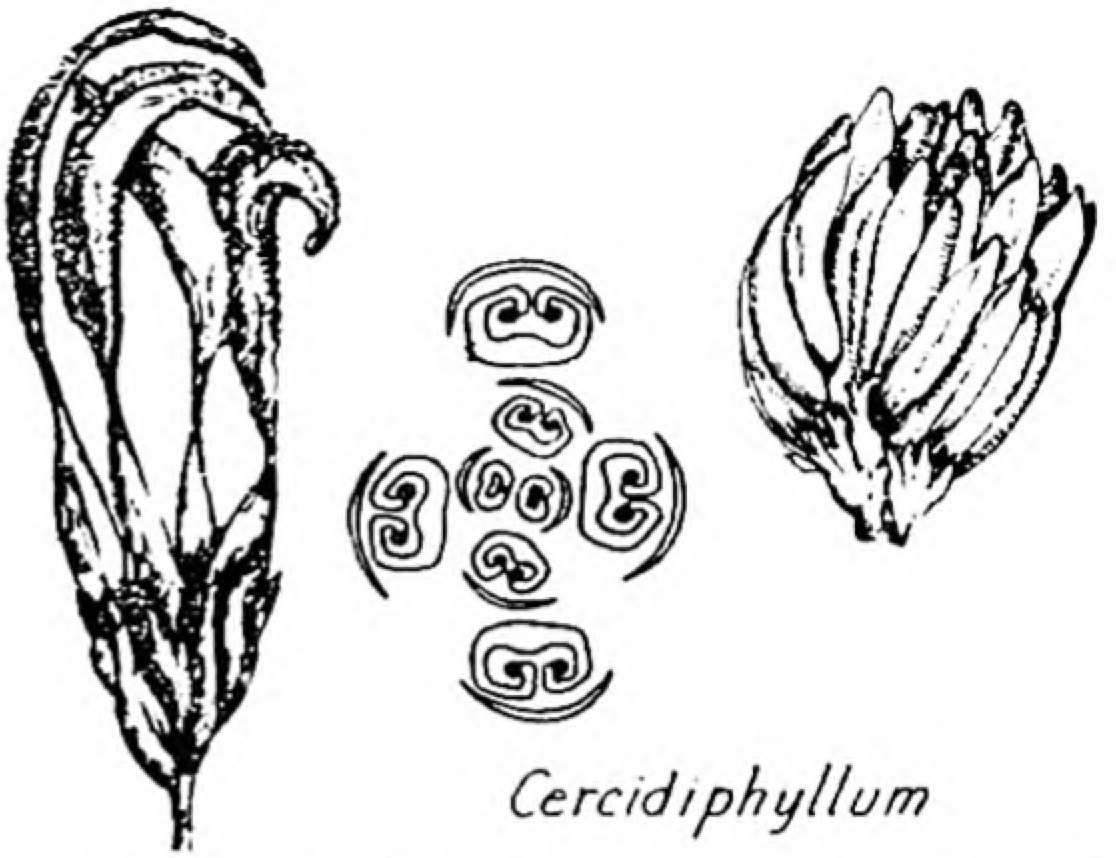


Fig. 8. *Cercidiphyllum*. At left: group of female brachyblastic gonoclads (conventional 'flowers'); in centre: cross sections of the so-called pistils or 'carpels' with 'dorsal' groove (ultimately becoming a suture) opposite the bract; at right: group of brachyblastic androclads, each consisting of a cluster of stalked (=stamens) subtended by a bract (compare Fig. 1b).

The interpretation of the reproductive organs of *Cercidiphyllum*, given by Melville (1963, 1969) is much nearer the mark: each bracteated male or female unit is explained *as a single complex* *gonophyll*. My to some extent concurrent description is: each unit is *an only slightly modified gonoclad-stegophyll combination*. I agree with Melville as far as the interpretation leads to the conclusion that the floral region of *Cercidiphyllum* is in a still very primitive 'pre-floral' stage. In other respects he rather confuses the issue, e.g., by attributing a very complex mode of branching to his putative, ancestral female reproductive with a 'reverse' orientation of the ultimate gynophyll (Melville, 1969, p. 167, Fig. 19), whilst calling the topologically corresponding male structure a simple androphyll (apparently with a 'normal' orientation !). I, therefore, do not agree with Melville (l.c., p. 167) that the arrangement of the female organs 'is not suitable for condensation into a compound ovary." It is in fact an evolutionary phase in the semophylesis of the gynoecium which may lead to several types of compound ovaries as we shall see. Each male reproductive unit consisting of a bunch of stamens in the axil of a bract is of course a condensed PA (=androclad) with its stegophyll and the compound structures (the floral region of each fertile male branch) a, still rather primitive, male anthocorm. The topologically corresponding ovuliferous structure, usually called a 'pistil' or carpel', *is also subtended by a bract* and must, therefore, either be *a slightly modified but still 'naked' gynoclad* that developed a stylar extension with stigmatic zones (in this case it is a 'phalanx' of concrescent monogyna as found in some Pandanales such as *Sararanga* and in Centrolepidaceae (compare Meeuse, 1965, p. 143, Fig. 10, p. 145, Fig. 11, top, and the text figure 7), or it is a monomerised gynoclad. This needs looking into.

Conceivably, a cyclisation of such gynoclads followed by their lateral coalescence and a longitudinal adnation of the the edges of the bracts to the ovuliferous parts would produce a plurilocular, 'syncarpous' ovary with as many 'style-branches' as there are locules *and with parietal' placentation* (compare also Meeuse, 1966, p. 165, Fig. 15a). Individual adnation between a bract and a gynoclad would produce an apocarpous gynoecium and variations of the theme (with or without participation of the principal floral axis) may lead to a compound pistil with a common style, or one with laminal placentation or with protruding placental ridges or 'free central' placentation of the centrospermous type. *Euptelea* bears androgynous structures (see Fig. 2. A^2 and Fig. 9) which have hitherto always been referred to as 'flowers' even by Melville (1963) although he showed that anatomically the so-called flower is of the nature of what I would call a single ambisexual gonoclad subtended by the single bract of the 'flower'. This is, metaphorically speaking, one of the many 'trials and errors' in floral evolution: a conversion of initially unisexual gonoclads into androgynoclads by the partial replacement of the more terminal MAs of an androclad by MGys or vice versa, which process has something to do with the advent of entomophily. This is a fairly recent phylogenetic development, first becoming manifest in the conventionally gymnospermous Chlamydospermae, so that the occurrence of ambisexuality of gonoclads in early angiospermous taxa is clearly a derived condition in respects of unisexual gonoclads, not a primary condition (an 'androgynophyll' as Melville has it). At any rate, the stalked 'pistils' are ecarpellate and represent monogyna i.e., chlamydote (arillate) ovules of practically the same type as found in e.g., *Gnetum* and *Welwitschia.* These pistils are the direct derivatives of the ovuliferous cupules of Mesozoic, presumably caytonealean—corystospermaceous cycadospid gymnosperms—one of the direct phylogenetical links between gymnospermous groups and early angiosperms.

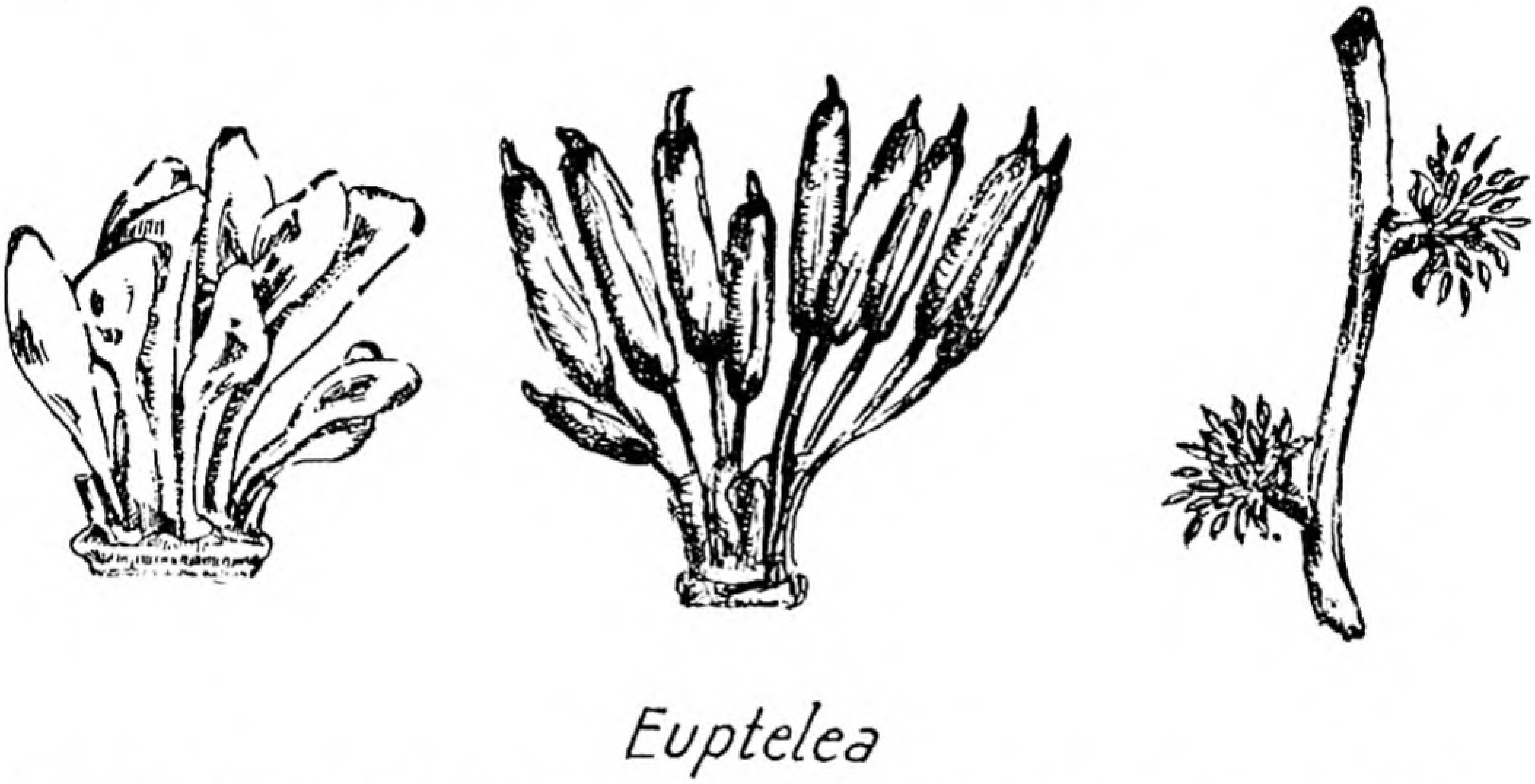


Fig. 9. *Euptelea.* At right: fertile twig in anthesis (slightly reduced); in centre: androgynoclad (=conventional 'flower' at anthesis; MGys still very small (enlarged); at left: the same after anthesis, stamens have fallen off and the one-ovuled semi-mature pistils have grown out (enlarged).

A recent study of the ontogeny of the reproductive region of *Euptelea* by Endress (1970) does not plead against the interpretation of the so-called carpels (=pistils) as primitive monogyna; the vascular anatomy is in any event not necessarily different from that of a pistil that is a direct cupule homologue (Meeuse, 1964, 1966). To my mind, the most important conclusion is that there is not the faintest indication of a vestige of a perianth. In my interpretation of the conventional 'flower' of *Euptelea* as a single androgynoclad supported by its bract *there can of course be no question of any perianth.* Other explanations, if based on the classical floral morphology, would require the derivation of the floral region of the undoubtedly primitive *Eupteleaceae* from a primitive (ranalean) type of 'bisexual' flower, with an apocarpous gynoecium of several pluri-ovulate follicular pistils ('carpels') with a ventral suture, and with a number of perianth lobes, involving as much reduction and oligomerisation that this is utterly paradoxal and indeed absurd. Why could a condition found in gnetalean taxa not have survived in putative, primitive angiospermous cycadopsids? The answer is of course that the conventional flower theory does not admit the possibility of the occurrence of reproductive regions in Angiosperms which are not interpretable as monaxial 'flowers'. I repeat that the alternative has its merits; it qualifies (*by consensus*) primitive *Euptelea* as a missing link.

A more detailed discussion of the floral morphology of these and of other primitive taxa in the light of this interpretation will be the subject of a forthcoming paper.

INTERPRETATION OF THE FLORAL REGION OF THE PIPERALES.

The conclusion can be drawn that a rather similar interpretation can be given of the floral region of the Piperales (see Fig. 2. A1). Clues are: the absence or the presence of bracts or 'perianth lobes', the presence of one-ovuled pistils, and the frequent occurrence of unisexual reproductive structures in some taxa, but also a tendency towards ambisexuality (as in Chlamydospermae and *Euptelea*). The relative degree of advancement is more or less: Chloranthaceae→Piperaceae→Saururaceae, but some characters may be more advanced in the reverse order.

Chloranthaceae.—The so-called 'flower' of *Chloranthus* is interpreted by Melville as a single androgynophyll. Translated into my terminology this would mean a single ambisexual gonoclad (with subtending bract) reduced to one female element (MGy) and a single male element (or at least very few MAs). The peculiar structure of the laminar microsporangiate organ of *Chloranthus* pleads against the idea of the reduction of the MAs of the androgynoclad to one (as in *Sarcandra*), but considering that the family is very primitive (the wood anatomy, especially that of *Sarcandra*, certainly points that way) we might speculate on the possibility of a comparison with the male reproductive organs of the gnetalean type (such as those of *Ephedra*) which may possess more than four pollen sacks. Swamy (1953) believes that the androecium of *Chloranthus* is complex and consists of three, usually more or less completely coalesced, stamens; if this is indeed the case, the androgynoclad of *Chloranthus* has retained one MGy and three MAs. In other genera (*Ascarina*, *Hedyosmum*) the stamen bears a 'normal' bithecal anther. Apart from a more advanced oligomerisation, the condition in *Sarcandra* (and *Chloranthus*?) is essentially comparable to that found in *Euptelea*. The diclinous unisexual reproductive structure of the genera is an excessively oligomerised, unisexual gonoclad reduced to a monogonon plus bract.

Piperaceae.—The situation in the 'bisexual' representatives (*Piper*, etc.) is not very much different from that found in the Chloranthaceae such as *Sarcandra*. The conventional 'flower' of *Piper* is, then, simply an androgynoclad reduced to a few MAs and one sessile MGy, and a whole 'catkin' is a modified anthocorm. A unisexual version is essentially the same as in the chloranthaceous genera *Ascarina* and *Hedyosmum*.

The Saururaceae and the Lactoridaceae, on the other hand, have floral structures (the conventional 'flowers') of a more complex nature which are radially symmetric. Any comparison with the first two families must not be based on the homologisation of the functional biological entity (the so-called flower), but on the possible indentity of elements with an anthocorm or parts thereof. Melville (1969), when discussing the Lactoridaceae gave two alternatives for the morphological interpretation of the floral region of this monotypic family. But I prefer the one which assumes a complex structure.

Each perianth lobe is, to my mind a bract subtending a 'mixed' gonoclad, all coaxial gonoclads having become laterally connate. The pistil is of the type of the Piperaceae (in the last case of the more ancient, pluriovulate type as also found in Saururaceae). The whole structure is a special, modified type of anthocorm reduced to a single subterminal whorl or three gonoclad-bract units which are longitudinally connate. The structural arrangement of the apetalous 'flower' of the Saururaceae suggests its identity with a single androgynous gonoclad with a few MAs and a few ecarpellate pistils. The outcome of these considerations is that the Piperales provide another example of the many 'evolutionary experiments' one encounters among the various primitive angiospermous groups. Apparently the 'mixed' androgynous gonoclad of the majority of the piperalean taxa (and of *Euptelea*) was, as a functional reproductive structure, not a very successful evolutionary achievement. In all more 'successful' and diversified 'modern' groups the flowers are either derived from anthocormoid structures with unisexual gonoclads, or from whole anthocorms with gonocladial androgyny by various evolutionary trends. Gonoclads sometimes became adnate to their subtending bracts (some Laurales) or to the anthocorm axis (=true floral axis) as in many Magnoliales, or they became laterally connate beyond the true floral apex (Winteraceae, Schizandraceae and Lactoridaceae, Meeuse 1971, in press).

FROM THE PIPERALEAN LEVEL OF FLORAL MORPHOLOGY TO THE RANALEAN 'FLOWER'

The monotypic family of the Lactoridaceae is considered to be 'intermediate' in characters in so far as it is sometimes placed in the Piperales and sometimes associated with the woody Ranales (compare, *e.g.*, Takhtajan, 1959, 1969; Engler-Melchior, 1964 and Weberling, 1970). The interpretation given by Melville, which I think is perfectly acceptable, if worded differently, is that only three 'mixed' gonoclad-bract units contributed to the origin of the 'flower' of *Lactoris*. Such androgynoclad-bract units exist in the true Piperales as we have seen, but there is a difference in that in *Lactoris* the units become longitudinally concrescent.

In the Magnoliales the number of 'units' contributing to the ultimate 'flower' was much greater than in the Lactoridaceae and the protomagnolean anthocorm must have resembled the conventional 'inflorescence' of the Saururaceae rather closely. The lack of any more intimate association of the gonoclads of the Piperales with their subtending bracts, and the absence of any trend towards adnation or connation of gonoclads, led the order into a dead-end street of floral evolution. This level was surpassed during the early evolution of the Mangoliales, Ranunculales, Berberidales, Laurales and some associated groups such as the Papaverales and Aristolochiales owing to the advent of various associations, adnations and concrescences leading to various floral types, as I shall discuss in detail elsewhere.

In the Winteraceae, Schizandraceae and Lactoridaceae, and presumably in most of the herbaceous Ranales, the gonoclads (unisexual and diclinous or androgynous) became laterally concrescent beyond the true floral apex as we have seen. In the Magnoliales there are two alternative interpretations: the stamens may be monandroids and the pistils gynonoids (by analogy with *Victoria*) but the flower can also be interpreted as formed by the lateral (longitudinal) adnation of androgynoclads to the anthocorm axis (=floral axis). The homology of the sterile floral organs is of course intimately connected with these interpretations.

The conditions in the lauralean lineage are fundamentally different (the Austrobaileyaceae may be exceptional). In this assembly, there are again two basic types of anthocorms, viz., the unisexual one and the type with gonocladial androgyny. Both the unisexual and the androgynous types subsequently underwent phylogenetic modifications, in several families by an adnation of the gonoclad to its subtending bract, in certain taxa among them associated with a broadening of the anthocorm axis into a receptacle or torus. In contradistinction to the condition in apparently all other Polycarpicae, in some of the taxa concerned the androgynoclads bore their MGys proximally and their MAs distally. In other lauralean forms the androgynoclads became curved inwards to become adnate to a broadened and concave receptacle e.g., Calycanthaceae. Barring the non-typically lauralean, but related and primitive, Austrobaileyaceae and Calycanthaceae, the *Monimiaceae* (with the accessory or peripheral groups of 'Amborellaceae', '*Atherosperm(at)aceae*', '*Trimeniaceae*' etc.) are undoubtedly the most primitive of this order. They are even predominantly diclinous or 'polygamous' which, in my opinion, is a case of primary= primitive unisexuality with incipient monocliny. This may also facilitate the interpretation, because the male and the female structures can be mutually compared on the basis of homotopy of their constituting elements. The most primitive androecial condition is that found in such forms as *Trimenia* and *Piptocalyx* (=family Trimeniaceae of many systematists). The 'stamens' are the single and almost entirely free MAs of a subterminal whorl of brachyblastic PAs (=androclads). A divergent evolution developed within the group if we presume that the immediate progenitors of the Laurales possessed androclads which were not so foreshortened in some groups, especially in the Monimiaceae— Monimioideae and incompletely in *Amborella*, the androclads became longitudinally adnate to their bracts (*Ephippiandra*, *Tambourissa*, *Monimia*), which ultimately led to a peculiar receptacle-shaped 'flower' as found in *Hedycarya*, *Levieria*; *Mollinedia*, and related forms) by the lateral concrescence of bracts and tepals. In other taxa, particularly in the Monimiaceae-Hortonioideae and Atherospermoideae and in all of the Lauraceae-centred group of families (Lauraceae, Hernandiaceae, etc.), the androgynoclads tended to become brachyblastic, whilst retaining only a single fertile MA or a few stamens, the remainder of the MAs having become reduced to the vascularised staminal appendages (which function as nectaries) or to staminodes.

These two divergent trends are paralleled by the female elements: The gynoclads, originally free as in the very primitive *Amborella*, usually became adnate to the tepals (which sometimes form a cup-shaped receptacle, which condition occurs in the *Monimioideae* usually in the same diclinous genera in which the male flowers are constructed in this way: *Ephippiandra*, *Hedycarya*, etc. ; the monogyna are occasionally sunk into the so-called receptacle as in *Tambourissa*); in other taxa they may have become oligomerised to a single MGy. In taxa with flowers derived from anthocorms with androgynoclads, the female elements (MGys) normally became oligomerised to a single pistil, at least in the more advanced families (not in Austrobaileyaceae and Calycanthaceae). The reduction of the androecium to a few 'stamens' with attached nectaries, or to a few stamens and staminodes, per gonoclad is almost always concomitant with monocliny and a valvular dehiscence of the anther loculi. The oligomerisation of the gynoecium must have been progressive because it is nowadays usually extreme, only a single and pseudo-terminal monogynon (=pistil) being normally present in the Lauraceae.

The Calycanthaceae are phytochemically a rather isolated group, but for various reasons a fairly close affinity with the Laurales is not at all unlikely. The Calycanthaceae provide another example of an early specialisation of the floral region, their entomophilous flower acting as a baited trap and temporary gaol. In the light of all preceding considerations the morphological interpretation in terms of a modified anthocorm must be as follows: the polliniferous organs are the MAs of centripetally incurved polyandrous androgynoclads adnate to the laterally broadened and concave receptacle. The pistils are, to my mind, the MGys of the proximal polygynous portions of the androgynoclads as indicated by the floral venation (compare Tiagi, 1963). The presence of *two* ovules per pistil is not necessarily a refutation of the interpretation of the pistils as monogynons: morphological structures from ovuliferous cupules are usually one-ovuled by progressive oligomerisation, but such cupules being initially pluriovulate a retention of more than one ovule is feasible, certainly in primitive taxa as the Calycanthaceae. The general floral morphology agrees fairly well with that of the primitive Monimiaceae, and the taxonomic place assigned to the Calycanthaceae in or very close to the Laurales by several recent taxonomists is altogether acceptable. The Austrobaileyaceae are not so well studied and all I can say for the moment is that in their floral organisation they may resemble the Magnoliaceae more than the Calycanthaceae, as I hope to publish elsewhere.

These examples show what I set out to prove, namely the emergence of clear taxonomic and phylogenetic relationships among the ranalean assembly from a postulated prototype which is approximately of piperalean affinity, provided the interpretation of the floral region is based on certain neomorphological tenets that can be concisely described as the postulate of the polyaxial flower. The likehood of divergent trends of evolution in different groups and lineages is still demonstrable in the recent representatives, particularly in the Laurales. The cognizance of divergent phylogenetic development clarifies the intranscendence of several floral characters in all terminal groups and will ultimately form the basis of our classification of these and of other angiospermous taxa.

SUMMARY AND CONCLUSIONS

The corollary of a survey of relevant data from various sources is that the Piperales are in several respects so primitive that, in spite of the conventional interpretation of their 'flowers' as phylogenetically highly advanced structures, the postulation of a generally low degree of morphological advancement seems to fit the evidence best. Alternative explanations, based on the neomorphological idea of a polyaxial anthocormoid floral region, have many consequences involving the floral morphology of several other taxa such as Magnoliales, Nymphaeales, Laurales, *Cercidiphyllum*, *Eupteleа* and *Trochodendron*. The interpretations given by the present author correspond in some essential points with those given by Melville in his gonophyll theory, but differ in several fundamental suppositions. An important conclusion reached by both these approaches to the interpretative floral morphology of the Angiosperms is that the conventional 'flowers' of numerous primitive taxa represent *single* modified units (bracteated polygonons or gonoclads, or gonophylls, respectively) and those of others, and especially of more advanced ones, *associated groups* of such units. The respective types of traditional 'flowers' of these two categories are of course not homologous, so that the cognizance of their morphological inequality cannot fail to have a considerable bearing upon our views concerning the phylogeny and the taxonomic relationships of the principal groups of the Flowering Plants, and upon their future classification.

However, the heterogeneity of the floral region is even greater owing to the divergent evolution and unequal degrees of advancement of the sporangiate floral parts, so that conventional 'pistils' and conventional 'stamens' may represent altogether different morphological categories of organs or of organ complexes. A pistil may be a modified single, ovuliferous cupule, it may be a modified gonoclad, or a fusion product of one or more gynoclad-bract units ('carpels', etc.). Androecial elements may be single monandrons of a group forming a polyandron, or the remnant of a reduced and oligomerised androclad, or perhaps sometimes the result of the phylogenetic and ontogenetic merging of a microsporangiate axis with its original subtending bract. Even the sex distribution varies, some groups consistently bearing only microsporangiate gonoclads and/or their macrosporangiate counterpart, and other ones, ambisporangiate (androgynous or 'bisexual') gonoclads. There must, accordingly, have been an appreciable, divergent evolution among the oldest Angiosperms leading to an early diversification. Several of these phylogenetic developments must undoubtedly have led to a rigidity of floral evolution, *i.e.*, to 'unsuccessful' evolutionary lines ending in as many 'terminal' groups which must, therefore, have retained a number of primitive features. One of the not unappreciable number of surviving groups of this kind must be the Order of the Piperales. The incidence of some singularly ancient characters in this order strongly suggests that this taxon is one of the oldest among the living Angiosperms. The floral morphology of the piperalean assembly of taxa is an example of an early specialisation which resulted in a phylogenetic dead-end street, but at the same time it gives us a lead when, in our quest for the ancestry and the early evolution of the Flowering Plants, we attempt the reconstruction of other, more successful evolutionary developments among protangiospermous forms.

REFERENCES

BAILEY, I. W., AND B. G. L. SWAMY. 1948. *Amborella trichorpoda*, a new morphological type of vesselless dictotyledon. *J. Arnold Arbor.* **29**: 245-254.

BREWBAKER, J. L. 1967. The distribution and phylogenetic significance of binucleate and trinucleate pollen grains in the Angiosperms. *Am. J. Bot.* **54**: 1069-1083.

CARLQUIST, S. 1964. Morphology and relationships of Lactoridaceae. *Aliso* **5**: 421-435.

——. 1970. Towards acceptable evolutionary interpretations of floral anatomy. *Phytomorphology* **19**: 332-362.

CRONQUIST. A. 1968. *The Evolution and Classification of Flowering Plants*. Boston.

DAVIS, G. L. 1966. *Systematic Embryology of the Angiosperms*. New York.

EAMES, A. J. 1961. *Morphology of the Angiosperms*. New York, Toronto, London.

EMBERGER, L. 1968. *Les plantes fossiles dans leur rapports avec les végétaux vivants. (Eléments de paléobotanique et de morphologie comparée)*. 2me ed. Paris.

ENDRESS, P. 1970. Gesichtspunkte zur systematischen Stellung der Eupteleaceae (Magnoliales). Untersuchungen über Bau und Entwickelung der generativen Region bei *Euptelea polyandra* (Sieb. et. Zucc.). *Ber. Schweiz. bot. Ges.* **79:** 229-278.

Engler, A. 1964. *Syllabus der Pflanzenfamilien.* (12th ed.) H. Melchior (Ed.) II. Band. Berlin.

Erdtman, G. 1952. *Pollen Morphology and Plant Taxonomy. Angiosperms.* Stockholm.

——. 1969. *Handbook of Palynology. An Introduction to the Study of Pollen Grains and Spores.* Copenhagen, New York.

Heel, W. A. van 1966. *Morphology of the Androecium in Malvales.* Thesis Leyden, 1966 and Blumea. **13:** 177-394.

——. 1969. The synangial nature of pollen sacs on the strength of 'congenital fusion' and 'conservatism of the vascular bundle system', w. sp. ref. to some Malvales. *Kon. Nederl. Akad. Wetensch., Proceed.,* **C 72:** 172-206.

Hegnauer, R. 1962-69. *Chemotaxonomie der Pflanzen.* Basel, Basel and Stuttgart. Vol. I-V.

——. 1966. Aucubinartige Glukoside. Ueber ihre Verbreitung und Bedeutung als systematisches Merkmal. *Pharm. Acta. Helv.* **41:** 577-587.

——. 1969. Chemical evidence for the classification of plant taxa. In *Perspectives in Phytochemistry.* J. B. Harborne, and T. Swain (eds.). London, New York.

Heinsbroek, P. G., and W. A. van Heel. 1969. Note on the bearing of the pattern of vascular bundles on the morphology of the stamens of *Victoria amazonica* (Poepp.) Sowerb. *Koninkl. Nederl. Akad. Wetensch. Amsterdam, Proceed. ser. C,* **72:** 431-444.

Hutchinson, J. 1969. *Evolution and Phylogeny of Flowering Plants. Dicotyledons: Facts and Theory.* London, New York.

Kuprianova, L. A. 1967a. Apertures of pollen grains and their evolution in Angiosperms. *Rev. Palaeobot. Palynol.* **3:** 73-80.

——. 1967b. Palynological data for the history of the Chloranthaceae. *Pollen Spores* **9:** 95-100.

——. 1969. On the evolutionary levels in the Morphology of pollen and spores. *Pollen Spores* **11:** 333-351.

Lemesle, R. 1946. Contribution à l'étude morphologique et phylogenetique des Euptéléacées. *Ann. Sci. Nat., Bot.* **7:** 41-52.

——. 1955. Contribution à l'étude de quelques familles de Dicotyledones considerees comme primitives. *Phytomorphology* **5:** 11-45.

Mayr, B. 1969. Ontogenetische Studien an Myrtales-Blüten. *Bot. Jahrb.* **89:** 210-271.

Meeuse, A. D. J. 1961a. The Pentoxylales and the origin of the Monocotyledons. *Koninkl. Nederl. Akad. Wetensch. Amsterdam, Proceed., ser. C* **64:** 543-559.

——. 1961b. Marsileales and Salviniales—'Living fossils'? *Acta bot. neerl.* **10:** 257-260.

——. 1963. Stachyospory, phyllospory and morphogenesis. *Advanc. Front. Pl. Sci.* **7:** 115-156.

——. 1964. The bitegmic spermatophytic ovule and the cupule: A reconsideration of the so-called pseudo-monomerous ovary. *Acta. bot. neerl.* **13:** 97-112.

——. 1965. Angiosperms—past and present. Phylogenetic botany and interpretative floral morphology of the flowering plants. *Advanc. Front. Pl. Sci. (Spec. Vol.)* **11:** 1-228.

——. 1966. *Fundamentals of Phytomorphology.* New York.

——. 1967. Again: The possible growth habit of the early Angiosperms. *Acta. bot. neerl.* **16:** 33-41.

——. 1970a. The descent of the flowering plants in the light of new evidence from phytochemistry and from other sources. I. General discussion. *Acta. bot. neerl.* **19:** 61-72.

——. 1970b. The descent of the flowering plants in the light of new evidence from phytochemistry and from other sources. II. Suggestions for a holotaxonomic major classification. *Acta bot. neerl.* **19:** 133-140.

Melville, R. 1962. A new theory of the Angiosperms flower. I. The gynoecium. *Kew Bull.* **16:** 1-50.

——. 1963. A new theory of angiosprems flower II. The androecium. *Kew.* **17:** 1-63.

——. 1969. Floral structure and evolution. I. The Magnoliales. *Kew Bull.* **23:** 133-180.

Metcafe, C.R., and L. Chalk. 1950. *Anatomy of the Dicotyledons.* Oxford.

Moeliono, B. M. 1966: *De caulomatische oorsprong van zaadknoppen bij Caryophyllaceeën en Primulaceeën.* Thesis, Univ. of Amsterdam, Amsterdam.

——. 1970. *Caulinary or carpellary placentation among Dicotyledons (Axis-borne versus leaf-borne ovules).* Pts. I and II. (in the press) Assen.

Money, L. J., I. W. Bailey, and B. G. L. Swamy 1950. The morphology and relationships of the Monimiaceae. *J. Arnold Arbor.* **31:** 372-404.

Muller, J. 1970. Palynological evidence on early differentiation of Angiosperms. *Biol. Rev.* **45:** 471-450.

Nast, G., and I. W. Bailey. 1945. Morphology and relationships of *Trochodendron* and *Tetracentron* II. Inflorescene, flower and fruit. *J. Arnold Arbor.* **26:** 267-276.

——. 1946. Morphology of *Euptelea* and comparison with *Trochodendron. J. Arnold Arbor.* **27:** 186-191.

Ozenda, P. 1949. Recherches sur les Dicotyledones apocarpiques. Contribution à l'etude des Angiospermes dites primitives. *Ecole. Norm. Supér. Paris, Publ. Laborat., Biol. II*: 1-183.

Raju, M. V. S. 1961. Morphology and anatomy of the Saururaceae. I. Floral anatomy and embryology. *Ann. Mo. bot. Gdn.* **48:** 107-124.

Swamy, B. G. L. 1953. The morphology and relationships of the Chloranthaceae. *J. Arnold Arbor.* **35:** 375-408.

Swamy, B. G. L., and I. W. Bailey. 1949. The morphology and relationships of *Cercidiphyllum. J. Arnold Arbor.* **30:** 187-210.

——, and ——. 1950. *Sarcandra,* a vesselless genus of the Chloranthaceae. *J. Arnold Arbor.* **31:** 117-129.

TAKHTAJAN, A.L.1959. *Die Evolution der Angiospermen*. Jena.

——. 1969. *Flowering Plants — Origin and Dispersal*. Edinburgh.

THORNE, R. F. 1968. Synopsis of a putative phylogenetic classification of the flowering plants. *Aliso* **66:** 57-66.

TIAGI, Y. D. 1963. Vascular anatomy of the flower of certain species of the Calycanthaceae. *Proc. Indian Acad. Sci.* B, **58:** 224-234.

VIJAYARAGHAVAN, M. R. 1964. Morphology and embrology of a vesselless Dicotyledon—*Sarcandra irvingbaileyi* Swamy and systematic position of the Chloranthaceae. *Phytomorphology* **14:** 429-441.

WEBERLING, F. 1970. Weitere Untersuchungen zur Morphologie des Unterblattes bei den Dikotylen. V. Piperales. *Beitr. Biol. Pfl.* **46:** 403-434.

ZIMMERMAN, W. 1959. *Die Phylogenie der Pflanzen*. (2. Aufl.). Stuttgart.

ALTERNATIVE GOALS FOR FLOWER MORPHOLOGY

By Richard I. Greyson

Department of Plant Sciences, University of Western Ontario, London, Canada

Abstract

In an attempt to make insights on flower development and structure available to a wider spectrum of biologists, a number of alternatives to traditional approaches are suggested. Legitimate morphological study could include (a) thorough quantitative analysis of organ development from initiation to maturity, (b) careful determination of the extent and pattern of cell division and elongation, (c) increased study of genetic and other teratological forms. In addition to their traditional phylogenetic interest, we feel that flower morphologists should place considerably more emphasis on ontogeny.

Angiosperm flower morphology is often restricted to questions concerning phylogeny or argumentation on the putitive homologies of the various organs. Rarely does one expect flower morphology to concern itself with the developmental vision that the flower is an end product, or better, a near terminal phase in an immensely intricate and beautiful web of interacting influences that have their beginnings in the flower meristem, and their culmination as the organs wither and die or abscise. That the flower has been ignored from a developmental standpoint can be inferred from the omission of any discussion of it in a recent review of initiation of organized development in plants (Torrey, 1966).

The rationale of some developmental botanists for ignoring the flower has been that the flower is too variable and complex. A possibly greater deterrent that confronts the developmental botanist, however, is that despite the immense body of descriptive data prepared over the years by flower morphologists, very little is useful or interpretable by botanists seeking to explore the mechanisms by which organogenesis and differentiation proceed. Considering the 1/4 million species of angiosperms that are extant, only a handful of careful and thorough descriptive treatments of flower ontogeny exist.

It is the purpose of this paper, then, to outline a valid and potentially fruitful area of study which can legitimately be called flower morphology and which can be an alternative intellecutal position for those who wish something more than what is offered by the traditional approaches. Flower morphology, if given some additional goals, might then regain some of its lost status so that it can again complement in a very natural way the disciplines that relate to other levels of flower structure and function.

It is not our intention to suggest that the problem of homology is an unimportant consideration. Obviously, when making comparisons it is essential to have a clear understanding of the degree to which the items one wishes to compare are similar or dissimilar. Homology, however, as suggested by Sokal (1962) and Sattler (1966) should be defined in operational and non-intuitive terms. Neither is it our intent to suggest that the working out of phylogenetic relationships is an unimportant goal. It is, however, not necessarily the ultimate goal of morphological study; and in the absence of a good fossil record (as is the case in the angiosperms) speculation can prove to be a somewhat unrewarding exercise. A more thorough interaction between morphology and those insights gained from genetics, physiology and developmental biology might prove ultimately more powerful in unravelling phylogenetic trends. (Wardlaw, 1952).

What, then, should be the premises, basic assumptions, and goals of the developmental approach to morphology? The following items suggest themselves.

1. The observations of form and structure of the flower of any of its organs are the primary data of flower morphology. It must, however, be thoroughly appreciated that form is produced through a progression of forms from an embryonic tissue. The form at any stage is the end product of a large number of cellular, physiological and biochemical expressions of the genome interacting within an environmental situation. Morphology, then can be defined as a body of knowledge relating to a description of that form as it develops.

2. A legitimate activity of morphology, therefore, would be the careful and precise description of the changes and developments of the form through time. These could encompass changes in shape, size, growth pattern, etc.

3. Description will of necessity begin with qualitative interpretations of the changes and of the mature form, but ultimately the goal of careful quantitative description should be realized. Where possible, changes and final structure should be expressed in terms of the cellular constituents of the organ; i.e., number and sizes of cells, regions of division and elongation or enlargement, and patterns of orientation of division planes.

4. When comparisons are to be made between the forms or developmental patterns of two or more organs, clearly operational and carefully defined bases of homology should be applied.

5. Morphological observations, like other investigations, are basically sampling exercises in a population of inherently variable material and therefore must be treated as such. Reports should include information on sample size, range of variability and where appropriate, statistical tests should be applied.

It is appropriate at this point to distingiush morphology from other peripheral but related areas of interest. Morphology, if properly oriented, should be a clearly viable and important area of plant science that would be useful for a wide variety of biologists in addition to those interested in more classical considerations. As a body of primarily descriptive data it could be quite distinct; but, because of the interest of the researcher, might often overlap with *morphogenesis* which has as its goal the elucidation of the factors underlying the form and its changes. Because of the manner of its presentation, morphological information would be readily interpretable and useful to the physiologist—a situation that rarely exists at present. Possibly more difficult to delimit is the area of *anatomy*, which deals with the tissues and cells of the organs. Since traditional flower morphology rarely makes a precise distinction here perhaps none is necessary in this instance.

It is to be emphasized that the hallmark of good morphology will be careful description. In a qualitative way this has always been true of good flower morphology, but we would add that it is now opportune to begin the description of the ontogeny of flower form in a careful and in a reproducible quantitative manner. Few model studies of flowers are available but numerous quantitative analyses of other organs can serve as examples of the work that could be done. Avery's (1933) study of the tobacco leaf, while including observations that go beyond the purely morphological, continues to be used by researchers as a basis for their studies. Many other models of careful quantitative analyses of other organs might also be given.

Let us now turn to some of the specific kinds of flower study that could be explored as perfectly valid morphological investigations and which would utilize the technology and techniques that morphologists normally employ. I consider the following items as being of considerable priority at the present time.

1. Thorough quantitative analysis of the developmental stages of organ growth from initiation to maturity are needed. Information from organs such as lengths, widths, regions of growth, changes in shape etc. if collected at different stages and portrayed on a time scale and analysed in a variety of ways (i.e., graphically, allometric relationships, rate studies, etc.,) would provide excellent insight into the total pattern of organ development. If to this we are able to add details of important cytological events, a rather comprehensive view of flower ontogeny will emerge. Considerable care must be taken in choosing the specimens for study and in preparing the timed collection of material (preferably grown under uniform and/or defined culture conditions). Observations could be made in part from fresh material, but more often would entail the collection of data from serial sections. Williams' (1966) study of the growth of the primary shoot and inflorescence of wheat and that of Nicholls and May (1964), while containing much data of primary interest only to the physiologist, do serve as models of the kinds of careful quantitative morphological study that are quite within the technology of the morphologists and in fact quite within the realm of flower morphology. The information obtained is of interest in itself, but as well is of considerable interest and value to those who have other interests and other technologies. The only restrictions on the usefulness of these observations by physiologists and others would be that the conditions under which the data was collected be given as well as full particulars on the number, precision and accuracy of all observations. It is important, then, that a morphologist, far more than heretofore, presents details of the number and variability of his observations.

2. Another very much needed and related study is to describe carefully the growth changes that do take place in flower organs in terms of the relative contributions of cell size and cell number to the growth patterns described. Many studies, possibly similar to those of Kaufman et al. (1965) on the growth of internodes of the *Avena*, or that of Greyson and Tepfer (1966) on the stamen filament of *Nigella*, might be performed. Again considerable information can be obtained from serial sections of organs at different stages of growth. For those organs that are large or for those which are primarily growing in one dimension

such as filaments, gynophores, styles, etc., considerable information can be obtained from observations of the epidermis of fresh material. In addition, information on the relative contributions of cell size and number to growth can be obtained by careful study of macerated preparations (Sunderland, 1960).

3. A description of the meristematic patterns within the organs is also woefully lacking. Far too often casual references are made to qualitative estimates of the location and extent of intercalary growth of these determinate organs. The complexities of cell cycle, however, caution us against making hasty generalizations about mitotic activity from a few observations from a small collection of slides. Examples of analyses of intercalary growth of organs are those of Jacobs (1947), Peterson and Cutter (1969), and Fisher (1970).

4. One can probably agree with Carlquist (1969) and Meeuse (1966) that the study of teratological specimens and genetically determined variability is of little use in answering evolutionary questions about flowers. If, however, our interest is in an appreciation of flowers as they develop and exist at present, then descriptions of developmental patterns which differ slightly from each other due to genetically determined mechanisms could be most informative. A great wealth of genetically determined modifications are known and available for study, but alas, few morphologists seem to appreciate this potential. The work of Posthlethwait and Nelson (1964), Stebbins and Yagil (1966) and Mathan and Cole (196 4) are examples for those interested in using mutants in the investigation of morphological problems. Considerable interest in teratological forms existed at the turn of the century, and probably, given the goal of phylogenetic description at the time, lost considerable favour among morphologists. Perhaps as we orient our morphological questions more directly to ontogeny a revival of interest in these deviant patterns might be expected.

Numerous other questions in addition to the four above could be cited as valid goals for the flower morphologist. Some will be of interest primarily to the morphologist and those interested in form. Others will be more oriented towards related disciplines of morphogenesis, cellular differentiation, and physiology. This should not disturb us. Flower form does not exist in a vacuum but at every stage is an expression of a host of interrelated and interacting genetically determined and environmentally modified physiological processes. The arbitrary decision to restrict morphology to one of those processes or end products is that of the morphologist, not that of the plant. Further, the realization that flowers as they exist possess information of evolutionary significance does not allow us automatically to infer the significance of that information. Nor does the presence of phylogenetic meaning preclude other kinds of significance and interest on our part. Perhaps flower morphology as a body of information and methods might well serve a number of different kinds of goals.

REFERENCES

AVERY, G. S., Jr. 1933. Structure and development of the tobacco leaf. *Am. J. Bot.* **20**: 565-592.

CARLQUIST, S. 1969. Towards acceptable evolutionary interpretations of floral anatomy. *Phytomorphology* **19**: 332-362.

FISHER, J. B. 1970. Development of the intercalary meristem of *Cyperus alternifolius*. *Am. J. Bot.* **57:** 691-703.

GREYSON, R. I., AND S. S. TEPFER. 1966. An analysis of stamen filament growth of *Nigella hispanica*. *Am. J. Bot.* **53:** 485-490.

JACOBS, W. P. 1947. The development of the gynophore of the peanut plant, *Arachis hypogea* L. 1. The distribution of mitoses, the region of the greatest elongation and the maintenance of vascular continuity in the intercalary meristem. *Am. J. Bot.* **34:** 361-370.

KAUFMAN, P. B., SANDRA J. CASSELL, AND P. A. ADAMS. 1965. On nature of intercalary growth and cellular differentiation in internode of *Avena sativa*. *Bot. Gaz.* **126:** 1-13.

MATHAN, D., AND R. COLE. 1964. Comparative biochemical study of the allelic forms of gene affecting leaf shape in tomato. *Am. J. Bot.* **51:** 560-566.

MEEUSE, A. D. J. 1966. *Fundamentals of Phytomorphology*. The Ronald Press Co., New York.

NICHOLLS, P. B., AND L. H. MAY. 1964. Studies of the growth of the barley apex. II. On the initiation of the internode elongation in the inflorescence. *Aust. J. biol. Sci.* **17:** 619-630.

PETERSON, R. L., AND E. G. CUTTER. 1969. The fertile spike of *Ophioglossum petiolatum*. I. Mechanism of elongation. *Am. J. Bot.* **56:** 473-483.

POSTLETHWAIT, S. N., AND O. E. NELSON. 1964. Characterization of development in maize through the use of mutants. 1. The polytypic (Pt) and ramosa-1 (Ra-1) mutants. *Am. J. Bot*. **51:** 238-243.

SATTLER R. 1966. Towards a more adequate approach to comparative morphology. *Phytomorphology* **16:** 417-429.

SOKAL, R. R. 1962. Typololoy and empericism in taxonomy. *J. Theoret. Biol.* **3:** 230-267.

STEBBINS, G. L., AND E. YAGIL. 1966. The morphogenetic effects of the hooded gene in barley. *Genetics* **44:** 727-741.

SUNDERLAND, N. 1960. Cell division and expansion in the growth of the leaf. *J. expl. Bot*. **11:** 68-80.

TORREY, J. G. 1966. The initiation of organized development in plants. *Advances in morphogenesis* **5:** 39-91.

WARDLAW, C. W. 1952. *Phylogeny and Morphogenesis*. MacMillan and Co., London.

WILLIAMS, R. F. 1966. The physiology of growth in the wheat plant. III. Growth of the primary shoot and inflorescence. *Aust. J. biol. Sci.* **19:** 949-966.

Adv. Pl. Morph. 1972: 33-45

CONTEMPORARY THOUGHTS IN PLANT MORPHOLOGY AND A NEW HYPOTHESIS OF THE BASIC MECHANISM OF MORPHOGENESIS

BY K. PERIASAMY

Department of Natural Science, Kalaignar Karunanithi Govt. Arts College, Thiruvannamalai, Tamil Nadu, India

ABSTRACT

Classical morphology was based on the assumption of certain fundamental categories of organs, the modification of which could account for all morphological variations in plants. With the birth and growth of genetics began the study of morphogenesis and this was also looked upon as a field related to classical morphology. However, morphogenesis, which aims at an objective interpretation of form in its own right, can have little relation with classical morphology, where conclusions are subjective, because of the lack of adequate scientific basis for the assumption of fundamental categories. Morphogenesis has hitherto been studied on the basic assumption that the nucleus is the organelle that regulates all morphogenetic phenomena but this approach has not solved its mystery. A critical appraisal of the development of the idea of nuclear control from the time of Lamarck, would indicate that like the fundamental categories of classical morphology, the concept of self-centred activity of the nucleus is not based on an objective and scientific concept of life. In fact, many biologists have recently begun to think that the environment of the nucleus—the cytoplasm—must be more important than the nucleus in morphogenesis. From the principle that in the organization of life into various biological levels a number of similar physiological and structural trends are seen in all the levels, we may hope to get an insight into the working of the cell from what obtains in the individual. If the brain and the body of the individual level are considered as counterparts of the nucleus and the cytoplasm of the cellular level, we may arrive at the hypothesis that the nucleus is a memory centre in the cell rather than a directive centre. For this, the cytoplasmic complex of the cell may be looked upon as responding to and reacting with its environment and fabricating molecules of single stranded DNA or RNA, with specific nucleotide sequence, for every specific environmental situation. These molecules could migrate into the nucleus for referring whether a memory of this is present in the nuclear DNA, and if so activate the memory. The memory would be the presence of a complementary of the nucleotide sequence of the cytomic molecule in the double stranded chromosomal DNA, and memory activation, the establishment of a complementary union between the two with resulting strand separation. Strand separation would be followed by transcription of the DNA code in mRNA, which then moves to the cytoplasm and engages in the translation of the code into a key polypeptide needed by the cell to set in motion the train of events or changes that the cell must follow in that particular cellular environment. When the cytome produces, as a result of an entirely new environmental situation, a molecule which has no memory in the nucleus, this molecule would continue to accumulate in the cell, move to other cells via the plasmodesmata or other ways, and may ultimately reach the sex mother cells. Here the molecule has the chance of getting incorporated during meiosis in the nuclear memory of the gametes and leading to a change in the morphogenetic pathway of the offspring. Chromosomal pairing and chiasma which result in genome changes are probably related to the incorporation of new memory molecules.

CLASSICAL MORPHOLOGY

Ever since Goethe published his "Metamorphosis of Plants", plant morphology had been of abiding interest to botanists in their attempts to penetrate into the basic phenomena that could explain and integrate the enigma of plant from in a comprehensive way. Although, just as any other scientific field, the field of plant morphology has to rely upon structural, ontogenetic and experimental studies for any valid conclusions, yet, an imaginative integration and comprehension of the results of various fields of study are what are needed for any worthwhile progress in this field. Early botanists like Goebel. Arber, Bower and others drew conclusions from studies that involved simple techniques at the organ and organism levels. As techniques became more sophisticated and laborious, and studies centred on the cellular and sub-cellular levels, the field of individual investigations became more restricted and the majority had neither opportunity nor the time to comprehend what was happening outside their own fields of studies. This naturally resulted in the dwindling of ideas of a comprehensive nature about plant morphology and an increase in ideas applicable to limited structures and phenomena.

The early morphologists supposed, without any understandable scientific basis. that the plant body was made up of only a few fundamental categories of organs, and all their ideas, inferences and conclusions revolved chiefly on how the varied structural features and organs of the plant body could be fitted in with these fundamental categories. In this regard, the position and vasculature of the organs were considered important in the early period, but later, ontogeny also became an important factor in morphological interpretations. Morphological interpretations often involved also comparisons between different plants, or comparative morphology, which was much concerned with evolution.

MORPHOLOGY AND MORPHOGENESIS

With the establishment of the chromosome theory of heredity, ontogenetic studies were approached from the angle of morphogenesis, as it was thought that the clue to what happened between the gene and the ultimate expression of the character controlled by the gene might be found out by carefully tracing the ontogeny of the organism and its individual organs. In this context, biologists began to consider morphogenesis as a special field or offshoot of classical morphology since both aimed at interpretation of form (Cusik, 1966).

Classical morphology has, however, never attempted to interpret form in its own right, but only the relationship of parts on the basis of the fundamental categories. Thus, in the interpretation of the morphology of a tendril or a thorn, classical morphology attempts to find out whether it is equivalent to a leaf or a stem, an axillary bud or a terminal bud, a stipule, etc., without going into the question of how the tendril form or the thorn form comes about. Similarly, in the interpretation of the flower and floral parts, classical morphology does not go into the question of how floral form and the form of its parts are brought about, but attempts equate the flower with a branch, and the floral parts with leaves, and simply conjectures the modifications necessary for transforming a leaf-like form into the dissimilar form of the floral parts.

Morphogenesis, on the other hand, does

not concern itself with the transformation of the supposed fundamental categories but tries to explain the development of all forms in plant and animal bodies, including the form of the fundamental categories of classical morphology. Morphogenesis is related to genetics, but this relationship is completely lacking in classical morphology. This is understandable because, at the time classical morphology began, and for a long time after that, the science of genetics was not born. In the comparatively short period that has elapsed since the birth of genetics, genetics has not been able to make its impact felt on classical morphology, and most of the classical morphologists neither make use of, nor are in need of genetical aspects for their interpretations. Thus, morphogenesis has little connection with classical morphology, although both are related to the broad field of morphology (Sinnott, 1966). Present day morphology may, therefore, be divided into two fields: classical morphology and morphogenetic morphology.

One group of morphologists would doubtless continue to follow the pathway of classical morphology, and indulge in interpretations which are purely subjective and do not lend themselves to an objective approach. For instance, in the interpretation of carpel morphology, Puri (1961, 1962) and others have always upheld the classical theory, whereas others like Bailey and Swamy (1951), Periasamy and Swamy (1956) and Swamy and Periasamy (1964) have given arguments in favour of a conduplicate primitive carpel. A perusal of literature on this subject would make it clear that in spite of the fact evidences are somewhat more in favour of the conduplicate concept, yet, as shown by Puri, they can always be brought within the framework of the classical theory. Thus, it appears that any argument in favour of one or the other could be made to fit in with the alternate theory *ad infinitum*, and nobody can say which is correct. For example, in a recent article Carlquist (1969) has challenged and doubted almost all the accepted concepts of floral morphology. This philosophical, rather than scientific state of affairs that obtains in the field of classical morphology, is due, essentially, to the fact that the whole domain rests upon the more imaginative and less scientific foundation of fundamental categories.

MORPHOGENETIC CONCEPTS

Morphogenetic morphology has a more exact role to play, as it aims at explaining how the form of the whole plant and its organs are brought about in relation to the genetical factors. Many biologists have attempted to unravel this mystery through various lines of approach, such as ontogeny, tissue culture, biochemistry, biophysics, genetics and cell physiology. For example, the author (Periasamy, 1965) from his ontogenetic studies on palm leaves, has distinguished three kinds of tissue growth in regard to cell size relationship. The three kinds are, (i) generative growth, (ii) perpetuative growth, and (iii) differentiative growth. In generative growth, there is a decrease in the average size of the cells; in perpetuative growth, there is no change in the average size of cells; and in differentiative growth, the average cell size increases. Although these have been found out with reference to the palm leaf, it is probable that they apply to all tissue growth in general and thus provide a concept for understanding certain aspects of growth phenomena. However, except for such limited concepts which apply to restricted fields of morphogenesis, there has been little progress in the understanding of the basic phenomena that underlie morphogenesis. Recent reviews of morphogenetic literature, like the book "Trends in Plant Morphogenesis" (Cutter et al., 1966) and Steward and Mott (1970), greatly emphasize this lacuna.

One concept that has become established on a firm foundation in regard to morphogenesis is that the nucleus as the organelle that contains the genes, is the most important structure that controls and influences morphogenesis. While the influence of the nucleus on morphogenesis is generally accepted, what is sadly lacking is the explanation of how the nucleus that contains all the genes of an organism is able to bring about the development of particular characters controlled by particular genes at particular locations on the body of the organism, and at particular times in the life of the organism. It is now generally thought that the nucleus can switch on certain genes and switch off others and by this device controls morphogenesis. The active genes are supposed to be located in the euchromatin, and the inactive genes in the heterochromatin. But the mechanism by which specific genes are switched on and activated, or switched off and repressed, is not yet known, and the operon, repressor, regulator concepts that have been postulated, still leave the basic mechanism that would account for their initial synthesis and activation unexplained.

The firm establishment of the idea of nuclear control over morphogenesis, after the discovery of the genetical fact of gene location within the nucleus, was due, in no less a measure, to the philosophy about life that has already become deep-rooted as a result of the widely accepted theory of natural selection. According to this theory, variations in organisms arise of their own accord without relation to the environment and the environment selects the naturally occurring variations. When it was known that the nucleus was the seat of genes whose variations alone could constitute heritable variations, it was naturally surmised that genetic variations should arise of their own accord in the nucleus. If thus, the genes could vary of their own accord, then the mechanism of morphogenetic regulation could also be self generated within the nucleus.

In this context, it would be worthwhile to make a reappraisal of the relationship between the basic philosophy of the theories of Darwin and Lamarck. The idea of Lamarck has been generally considered as quite different from that of Darwin, but a critical analysis would show that the two do not differ much. Both recognized and gave importance to the environment as the chief instrument in the evolution of organisms. The only difference between the two was the level of biological organization at which the environment was construed to act and lead to organic evolution. According to Darwin, the environment produced heritable modifications of populations, but according to Lamarck, the environment brought about heritable changes in the individual. In order to modify the individual, the environment has to act on the cellular level, but to modify the population, it is enough if the environment acts on the individual. Thus, the idea of Lamarck merely gave a wider scope and field for the environment than that of Darwin because, all levels higher than the level at which environment begins to act, would automatically come under its sway.

Without going into the details of the experimental proofs and other aspects that led to the rejection of Lamarck's theory, we may say that the rejection was but natural, considering the state of biological knowledge at that time. Whereas it was easy to perceive the influence of the environment on the individual, none could conceive how the environment could act at the cellular level. When we see that in spite of the great advances that have now been made in our knowledge about the sub-organismal levels, we have not yet been able to think of the action of the environment below the individual level, it is not surprising that it was so at the time of Lamarck. Lamarck's idea was based on something, the comprehension of which was far beyond the reach of his time, but the concept of Darwin, although it lacked much in regard to the mechanism of action of the environment, and was even erroneous in certain respects as we know now, provided an easy working hypothesis, which was well within the reach of the knowledge of his time.

The important outcome of the rejection of Lamarck and the acceptance of Darwin was the philosophy that the influence of the environment cannot be felt at the cellular level and that at this level, all the activities of life, including the production of variations, are self generated without relation to the environment. When the independently varying life and environment meet at the level of the individual, life thrives only where the variation in both are compatible to a certain extent, and otherwise life perishes.

When in the background of this philosophy, the genetic processes like deletion, duplication, inversion, translocation, mutation, etc., which result in genome changes were discovered, it looked but natural that no cause for these occurrnces could be found. Indeed, it was not even doubted that these as well as the other activities of the nucleus could be related to the environment, leave alone the environment of the organism, but even the immediate environment of the nucleus—the cytoplasm.

The fact that we have been unable to conceive how the environment could filter through the individual and act on the sub-organismal levels, is not a valid reason that this must be so. While the concept of self-centred activity of life at any level, could be in consonance with the vitalistic philosophy of life wherein physico-chemical or any other objective explanation of life is not necessary, such a concept cannot be in harmony with the philosophy of a mechanistic and objective concept of life, which may be said to be the very soul of the science of biology. It would be contrary to the basic tenets of science that any level, even in the physical world, leave alone the biological world, can exist and act independently of its environment. Indeed, even as the philosophy of fundamental categories has been inhibiting the field of classical morphology from reaching any objective conclusions, the philosophy of self-centred activity of the nucleus has been inhibiting the field of morphogenesis.

A Recent Trend in Morphogenetic Thought

A perusal of recent literature on morphogenetic thought indicates that biologists have begun to feel that the problem of morphogenesis cannot be solved on the basis of self-centred activity of the nucleus, and that the cytoplasm must be assigned a more important role, including the capacity to respond to external stimuli. The following extracts, given by way of example, amply illustrate this.

"It is clear that in the fomation of cell walls there is even greater scope for the regulatory control which is exercised by the surface of the protoplasm in response to external stimuli". —"Effects such as these, that can be multiplied for higher plants, are not alone susceptible to genetic or biochemical explanation without recognizing the additional directing influences exerted by factors in the immediate, organized environment in which the synthetic processes occur." "This sort of work leads to a concept of the living cell, genetically endowed with certain biochemical potentialities but controlled and integrated in its behaviour by essentially non-genetic (epigenetic) factors." —"the organization we designate as the protoplast must respond to exogenous stimuli which emanate from the external environment and from other organs and these may control or modulate the course of metabolism and development" (Steward, 1966).

"To be able to refer wall morphogenesis to the activity of a component of the protoplasm (other than the nucleus) makes a step forward, but it needs hardly be said that many more factors remain to be identified" (Heslop-Harrison, 1966).

Granting that we accept the concept of the environment influencing morphogenesis through the cytoplasm, still as stated by Steward (1966), "the point of impact and the mode of action of these stimuli and external factors is a cardinal problem".

LESSON FROM LEVELS OF BIOLOGICAL ORGANIZATION

In an attempt to find an answer to this cardinal problem, it would be of help to recognize that in the organization of life into various biological levels, there occur many structural and physiological trends that are significantly similar in all the levels. For example, the cell, and the individual, which represent two levels of biological organization, exhibit a number of common features, so that a multicellular organism has been looked upon by many as a republic of cells. As it is not necessary for our purpose here to go into the details of the many common trends that are observable in the levels of biological organization we may do well to take this as a general principle.

Since, in general, observations and inferences on higher levels are easier because the units are larger and do not need as much instrumentation and technical skill as the lower levels, we may, by applying our knowledge of a higher level, discern much about a lower level, and this would provide a basis for interpretations of the lower level. This is not only true of biological levels, but also of the physical and chemical levels, one famous example among the latter being the periodic table of elements, propounded on the basis of the chemical properties of elements, long before the protons and electrons were found out and whose configuration in the atom is the basic feature of the periodicity.

In the organization of biological levels, animals and plants are generally considered on a par, but it appears that there is much difference between the two in regard to organization above the cellular level. In the organization and elaboration of the individual form, plants have remained in a primitive state in which the higher potentialities of the cell have not come into play. For example, in the matter of coordination, plants have only the coordination through movement of chemical substances which involves the biochemical potentialities of the cells, and have not developed the mechanism of nerve conduction which involves the biophysical potentialites of the cell. This is probably related to the development of a rigid cell wall which, by acting as an effective shield and filter point of the environmental factors, restricted the need and scope for complex profoplasmic elaboration against the challenge of the environment. In the animals, the protoplast did not develop any such effective barrier against the environment but met the challenge by complex elaboration of the external form and internal coordination, utilising not only the biochemical but also the biophysical potentialities of the cell. In short, the plant kingdom appears to have withdrawn itself more and more from the environment by perfecting its protective shelter, while the animal kingdom has invaded more and more into the environment and conquered the elements of the evironment to its own advantage. Hence, in our attempt to fathom about the lower biological level—the cell, we may benefit more from what obtains in the animals than from the plants, but whatever insight we gain would be applicable to all cells.

As regards the problem of morphogenesis at the cellular level, when we look for the counterparts of the nucleus and the cytoplasm at the higher levels, we may well say that they are the brain (or coordinating machinery) and the body at the individual level, and the government and the public at the society level. In these, since the individual and the cellular levels are nearer to each other, we may hope to get an insight into the role of the nucleus within the cytoplasm of the cell, from the functioning of the brain within the body of the individual.

NEW HYPOTHESIS OF NUCLEAR ROLE

Although it cannot be said that we have succeeded in understanding the functioning of the brain fully, it is certain that it functions essentially like a computer in so far as its relation with the rest of the body is concerned. In this regard we know that the brain does not feel by itself and serves mainly as a storehouse and reference point of memories of the experiences of the body. If we look at the cell in this light, the function of the nucleus would also be to serve as a storehouse of the memory of all the experiences of the cytoplasm, and to which the cytoplasm refers every situation for proper guidance.

We know well that the cytoplasm alone comes in contact with the environment of the cell. The nucleus is always surrounded by cytoplasm and there is no known instance where the nucleus is not so surrounded. All cellular differentiation concerns only the cytoplasm and its surrounding glycocalyx, whereas the nucleus does not exhibit any significant morphological variation in the cells of an organism. These lead to the hypothesis that the cytoplasm or the cytome which is exposed to the cellular environment, has the capacity to follow suitable morphogenetic pathways in response to environmental stimuli, but by referring every crucial move to the nuclear memory and getting proper guidance. Such a hypothesis would, however, require an explanation of the nature of the memory and the reference processes.

MECHANISM OF MEMORY REFERENCE

Although much experimental work needs to be done in order to find out whether the above hypothesis is true, and if so the involved mechanism, an explanation may be advanced on the basis of existing knowledge.

We know that nerve conduction of

impulses to and from the brain is essentially electrical. Electrical conduction in the cell would require specific pathways that are insulated from the surrounding medium. The structures which qualify for such a pathway in the cell are the microtubules and the network of endoplasmic reticulum. The microtubules are supposed to be associated with intracellular movement. The ER pervades throughout the cytome and has connections with the plasma membrane on the exterior and the nuclear membrane on the interior, the nuclear membrane, in turn, being connected to the chromosomal genome within. These two structures are fitted to conduct electric impulses emanating anywhere in the cytome to the nucleus and vice versa. If this be the case, specific pathways of conduction may be obtainable in the microtubule system and the three dimensional network of the ER. It is also possible that the ribosomes which line the ER may also be involved in producing specific pathways, by virtue of the geometry of their alignment and other features.

In the generation of an electric impulse in the cell, we may assume two possibilities: 1. the membrane or microtubule system of the cell could be physically stimulated at specific places by environmental stimuli to send suitable impulses to the nucleus which then sends out appropriate signals to the cytome; 2. the complex material of the cytome may respond to environmental stimuli by producing chemical substances which activate the microtubule or membrane system to generate suitable impluses that are sent to the nucleus. However, from the fact that morphogenetic processes in the cell are not as rapid as could be expected by electrical involvement, it is more probable that a movement of substances from the cytome to the nucleus and vice versa, for which there is adequate evidence at present, is the major mechanism that is operating in the memory reference process. Since at the individual level, all these three mechanisms do exist as, 1. direct physical nerve stimulation, 2. chemical stimulation of nerve, and 3. chemical coordination through hormones, auxins, etc., it is probable that all these three operate at the cellular level also, the selection of the mechanism being related to the nature of the stimulus. As stated earlier, the last mechanism alone is common to both plants and animals, whereas the first two are seen only in the animals.

It is difficult for us to surmise the details of the working of the first two mechanisms, supposing they do exist, because our knowledge of the biophysical aspects of the cells is meagre. However, the biochemical aspects of the cell, applicable to both plant and animal cell, has now been extensively studied and, on the basis of this, it should be possible to postulate an explanation of how the nucleus could act as a memory centre through chemical means. Since the best understood biochemical activity in which the nucleus is also involved is the programme of polypeptide synthesis, this activity should be explainable in terms of the present hypothesis.

In the scheme of polypeptide synthesis that has now been deciphered, two aspects that have not been adequately accounted for, are the mechanism of specific gene or portion of DNA molecule activation in preference to others, and the mechanism of strand separation of the double stranded DNA for transcription. These unsolved aspects, in fact, provide a basis for an explanation of our hypothesis.

The material of the cytome, vastly

more complex than that of the nucleus, may be thought of as responding to or reacting with its environment and fabricating reaction products for every specific environmental situation. These products may be molecules of single stranded DNA or RNA with specific nucleotide sequences. These molecules which probably reach the size of cistron units, could then migrate into the nucleus for referring whether a "memory" of this sequence is already present in the double stranded, chromosomal DNA. For this purpose, we may assume that the memory of any molecule of cytomically fabricated nucleotide sequence would be the presence of a complementary sequence of this in one of the strands of the double stranded, chromosomal DNA. When such a sequence is present, as in all probabilities there should be, the cytomic molecule could establish a complementary union with it, thereby causing strand separation, which process may be considered as the mechanism of memory activation. The other strand of the DNA at the region of memory activation which would be similar in its nucleotide sequence to the molecule that migrated from the cytoplasm, will now be free to transcribe its code by complementary synthesis of mRNA. This mRNA would then migrate to the cytoplasm and guide polypeptide synthesis in conjuction with the ribosomes. This polypeptide would be the key substance that is needed by the cell to initiate or mediate in biochemical reactions and other processes that are necessary to set in motion the particular morphogenetic pathway the cell should follow in that particular environmental situation.

The duration of the memory activation and the consequent train of events may be controlled by other kinds of impulses emnating from the cytome, or more probably, memory activation by any one molecule is of a fixed span of time, after which the molecule becomes degraded, so that another molecule would be needed to keep the activation going beyond that time. This could take place, provided the cytome continues to experience the same specific environmental stimulus, in which case, the same memory activation molecule would continue to be fabricated by the cytome. Thus, the nature of the polypeptide and the duration of its synthesis become related to the cellular environment.

The "environment" would comprise both external and internal factors. Only the cells exposed to the outside world, would have the influence of the external environment acting directly on them, but in the internal cells, the environment would be the resultant of the influence of the surrounding cells.

Since the environment, as well as the cytome, are very complex entities involving numerous factors interacting among themselves, there may simultaneously occur several situations each one of which results in the production of a specific molecule, and thus there may be a regular train of many memory activating molecules moving into the nucleus. As the nucleus is the repository of the memory of all the essential experiences, which the cytome of the organism has had in the past history of the species, in every one of its cells from the zygote onwards, the cytome of any cell is not likely to experience anything new. Hence, the nucleus will have memory for almost all the memory activating molecules produced by every cell cytome in response to its environment. This would account for the orderly development and differentiation of an individual in a characteristic morphogenetic pattern, to attain the morphological features distinctive of its species.

Besides intracellular activation of memory, intercellular activation may also take place by the movement of memory activating molecules from one cell to another through the plasmodesmata or other pathways.

MECHANISM OF MEMORY REGISTRATION

While the memory reference process envisaged in the hypothesis may be explained as above, the explanation of a memory registering process in consonance with the hypothesis is more important. When, in response to a "new" environmental situation, the cytome fabricates a molecule for whose nucleotide sequence there is no memory in the chromosomal DNA, this molecule will not be able to activate mRNA synthesis and hence, the key substance or polypeptide needed for meeting with the new situation will fail to be produced. Therefore, the cell cannot follow any new pattern of morphogenesis or activity. When the cytome is thus incapacitated to follow a new course, it could probably switch on to the customary path which cannot be very different, and in this regard, intercellular memory activation may also be involved. Nevertheless, if the new situation persists, the new molecules will continue to be produced. While those molecules for which there is a memory in the nucleus would become dismantled following the establishment of a complementary union with DNA, the new molecules would continue to accumulate in the cell. When a number of cells subjected to the same conditions behave so, there will be a large accumulation of the new molecules, which then would migrate to other cells through the plasmodesmata or other pathways and ultimately reach the reproductive cells also. In this context, attention may be drawn to the presence of several hundreds of repeated nucleotide sequences in the cellular DNA (Bitten and Khone, 1969; Walker, et al. 1969), as it is tempting to surmise that these may represent the accumulation of molecules which are not represented in the nuclear memory.

It is possible that during DNA synthesis prior to cell division, the new molecule may be incorporated in the DNA. Such an incorporation would first involve the conversion of the single stranded, memory activating molecule, into a double stranded, memory registered DNA molecule. It is probable that the mechanics of mitosis do not favour the incorporation new molecules in the genome and hence a somatic departure from the genome spelled pathway of morphogenesis rarely occurs. On the other hand, the mechanics of meiosis may be more favourable for the incorporation of new molecules in the genome DNA, and the probability of incorporation may increase with increase in the numerical strength or amount of the new molecules that reach the sex mother cells. The incorporation of a new molecule in the genome DNA during meiosis, would make some of the gametes and the zygotes resulting from them different, with an altered morphogenetic pathway. Thus, we have here, a mechanism in which genome changes are related to the environment of the parents.

In the scheme of incorporation of a new molecule in the nuclear memory, arise the questions whether the new molecule is incorporated in the place of the nuclear DNA which it is intended to replace. If so how does the incorporation take place at the exact location? Or, could the new molecule be incorporated anywhere in the genome and if so what happens to the DNA portion which is the memory of the earlier morphogenetic pathway? Although these questions cannot be answered satisfactorily now, for the purpose of our hypothesis, it is enough if the new molecule is incorporated anywhere in the genome in order to alter the morphogenetic pathway, and the memory of the earlier pathway need not be deleted. Furthermore, we may surmise that chromosomal pairing and chiasma formation of meiosis, which lead to genome changes like deletion, duplication, inversion, translocation etc., may be related to the incorporation of new memory sequences. If this be so, these genome changes for which no cause has yet been found, would also be, in essence, due to the influence of the environment on the organism.

EXPLANATION OF SOME MORPHOGENETIC EVENTS

The stage at which a new memory comes into play will have an important bearing on morphogenesis, because, alteration of the morphogenetic pathway at certain crucial stages may result in significant changes, even if there is no change in the other stages. For example, taking the case of leaf ontogeny, there are in the whole programme, four crucial stages:

(i) Inception of the primordium; (ii) Cessation of the activity of apical growth; (iii) Initiation or differentiation of marginal initials; (iv) Cessation of the activity of the marginal meristem.

Any significant change in leaf morphogenesis will have to take place by alterations in these crucial stages, and the leaf type may be changed by altering anyone of these stages without any accompanying change in others. For instance, the transformation of a simple leaf into a compound leaf or vice versa can occur by a change in the disposition of the marginal initials. If the marginal initials that give rise to marginal growth form a continuous strip and function more or less equally, a simple leaf would result, but if alternating groups or initials become arrested, a compound leaf would result, although the other stages remain unchanged. A similar view has been expressed by Ramji (1967) with regard to the ontogeny of the venation pattern in the leaf of *Calophyllum inophyllum*.

The development of any character in an organism, including the manner of development of the character, means that the nucleus of the organism contains the memory for its initiation and sequence of development. The modification of the memory must take place by continued reactions of the cytome which result in the production of new molecules not represented in the concerned memory. For example, in a plant with simple leaf, the nucleus will necessarily have memory for the establishment of a continuous strip of marginal initials and the functioning of the initials more or less equally. If the cytome of the cells of the leaf primordium experience such conditions as would result in the fabrication of molecules that could lead to events which arrest alternating groups of initials, such a morphogenetic change could not occur because, these new molecules will have no memory in the nucleus, and hence, the cells cannot set in motion the altered morphogenetic pathway. Therefore, the primordium would continue to follow its customary pathway and develop as a simple leaf, although the totality of its environment is such as would make it develop into a compound leaf. However, if all the leaf primordia, or a majority of them, continue to be subjected to the new stimulus of the environment, the resulting new molecules would not only begin to accumulate in the cells of the primordia but also move to other cells. If thus the new molecules reach the microspore or megaspore mother cells, there is a likelihood of of the molecule getting incorporated in the nuclear memory of some of the gametes. In the offsprings that result from such gametes, the leaf primordia would develop into compound leaves.

Let us take another instance for explanation. In tissue culture studies, it is well known that the younger the embryos or primordia, the more difficult are they to culture, and the more complex the media they require. Even then they never attain their natural size but begin to differentiate and acquire their mature morphology while they are still comparatively very small. Several reasons have been ascribed for this, such as the one that the smaller the volume of the embryo, the larger is its relative surface, so that, certain essential substances or metabolites produced by the cells and needed for progressive growth and differentiation, easily diffuse out to the surrounding medium without being retained inside. According to the present hypothesis, the explanation would be, that the cells of the embryo experience a different environment in vitro, and hence, their cytomes react differently and produce molecules which have no memory counterpart in the genome. Therefore, the key polypeptides needed for development fail to be produced, resulting in metabolic blocks or shocks that affect all vital processes. The smaller the size and lesser the number of the cells, the more pronounced would be this effect, but the larger the size and more the number of cells, the lesser would be this effect because, the inner cells not directly exposed to in vitro conditions, will not be subjected to a markedly different environment from what they would have in vivo. Therefore the metabolism of the inner cells may proceed more or less normally. The surface cells also would be partially protected from the shock of having a new environment all around the cell.

Just as the morphogenetic events in the above two instances receive adequate explanation from the present hypothesis, other morphogenetic phenomena could also be accounted for.

CONCLUSION

The whole hypothesis advanced here may be explained by means of an example, again derived from another biological level. The morphogenetic programme of an organism is similar to the construction of a vast and complex building, in which each one of the numerous persons engaged in the construction has a blue print of the whole building, but proceeds with the work assigned to him by referring to that part alone of the blue print that concerns him. If anyone feels that an alteration in the portion assigned to him is desirable, he cannot do it of his own accord, but note it down, and get it incorporated in the blue print after proper consultations with the whole machinary of construction, before he could institute the alteration. The blue print, although it contains the basic information about the building, does not, and cannot by itself direct the construction.

The author is aware that the hypothesis is highly speculative and many more points remain to be answered, but believes that the assignment of the role of a memory centre, rather than that of a directive centre to the nucleus, would throw more light on aspects of morphogenesis that remain obscure now.

REFERENCES

BAILEY. I. W., AND B. G. L. SWAMY. 1951. The conduplicate concept of dicotyledons and its initial trends of specialization. *Am. J. Bot.* **38:** 373-379.

BRITTEN, R. J., AND D. E. KHONE. 1969. Repetition of nucleotide sequences in chromosomal DNA. In *Handbook of Molecular Cytology* North-Holland Publishing Co., Amsterdam, London. 21-36.

CARLQUIST, S. 1969. Toward acceptable-evolutionary interpretations of floral anatomy. *Phytomorphology* **19:** 332-362.

CUSIK, F. 1966. On phylogenetic and ontogenetic fusions. In *Trends in Plant Morphogenesis.* Longmans Green and Co., London. 170-183.

CUTTER, E. G. et al. (Eds) 1966. *Trends in Plant Morphogenesis.* Longmans Green and Co., London.

HESLOP-HARRISON, J. 1966. Morphogenesis at the sub-cellular level. In *Trends in Plant Morphogenesis.* Longmans Green and Co., London. 127-139.

PERIASAMY, K. 1965. Morphological and ontogenetic studies in palms. 2. Growth pattern of the leaves of *Cocos nucifera* and *Borassus flabellifer* after the initiation of plications. *Aust. J. Bot.* **13:** 225-234.

——, AND B. G. L. SWAMY. 1956. The conduplicate carpel of *Cananga odorata. J. Arnold Arbor.* **37:** 366-372.

PURI, V. 1961. The classical concept of angiosperm carpel: A reassessment. *J. Indian bot. Soc.* **40:** 511-524.

——. 1962. On the concept of carpellary margins. *Proc. Summer School Bot. Darjeeling.* 326-333.

RAMJI, M. V. 1967 Morphology and ontogeny of the foliar venation of *Calophyllum inophyllum. Aust. J. Bot.* **15:** 437-443.

SINNOTT, E. W. 1966. The geometry of life. In *Trends in Plants Mophogenesis.* Longmans Green and Co., London. 88-93.

STEWARD, F. C. 1966. Physiological aspects of organization. In *Trends in Plant Morphogenesis.* Longmans Green and Co., London. 3-26.

——, AND R. L. MOTT. 1970. Cells, solutes and growth: Salt accumulation in plants reexamined. *Internl. Rev. Cytol.* **28:** 275-370.

SWAMY, B. G. L., AND K. PERIASAMY. 1964. The concept of the conduplicate carpel. *Phytomorphology* **14:** 319-327.

WALKER, P. M. B., W. G. FLAMM, AND A. MCLAREN. 1969. Highly repetitive DNA in rodents. In *Handbook of Molecular Cytology:* North-Holland Publishing Co., Amsterdam, London. 52-66.

Adv. Pl. Morph. 1972: 46–50

FOLIAR SCLEREID PATTERNS AND THEIR ORIGIN

By T. Ananda Rao and O. P. Bhupal
Botanical Survey of India, 76, *L. C. Road, Calcutta*-14, *and*
G. C. Bose Biological Research Unit (*Bangabasi College*), *Calcutta*-9, India

Abstract

A resume on the progress in the studies on the foliar sclereid patterns has been presented in this paper. The recent literature on the cleared laminae of sclereid-bearing taxa throughout the phanerogams shows topographically three types of sclereid pattern: terminal, diffuse and mixed. In the first, sclereids are dispersed singly or in groups in direct contact with the tracheary elements of the veinlet endings. In the second, they occur freely in the leaf expanse and in the third, terminal and diffuse sclereids are dispersed in one and the same lamina.

Terminal Pattern

The prevalence of terminal sclereids and their great diversity of forms within a few genera has been the subject of a number of investigations, the most comprehensive being that on *Mouriria*, (Foster, 1946) and *Memecylon* (Rao, 1957). Schulze (1902) was the first to report sclereids in several species of *Boronia* of Rutaceae, at the veinlet endings. Subsequentely, terminal sclereids have been recorded in closely related and distinct families of seed bearing plants such as *Coryphadenia*, *Mouriri* (Morley, 1953a, b.) and *Memecylon* (*l. c.*) of the Melastomataceae. *Capparis orbiculata* Wall., *C. rotundifolia* Rottle., *C. verrucose* Jacq., *Niebuhria apetala* Dunn., *N. woodii* Oliv., *Boscia caffia* Sond. of the Capparidaceae (Rao, 1953, Rao and Mody, 1961); *Moutabea* of the Polygalaceae (Foster, 1947); *Hamamelis virginiana* L., *Exbucklandia populnea* (R. Br. ex Griff.) R. W. Brown, *Eustigma oblongifolium* Card. and Champ., *Rhodoleia teysmanni* Miq (Foster, 1947); in a few species of *Rhodoleia*, (Bhupal and Kundu, 1969) and *Disanthus cercidifolium* L., *Hamamelis mollis* L., *Liqudamber tricuspis* L. of the Hamamelidaceae (Bhupal, 1971); in a few species of *Boronella* of the Rutaceae (Foster, 1955a), *Hannao klaineana* Pierre and Engler of the Simarubaceae (Foster, 1956), *Cynometra cauliflora* L. of leguminosae (Rao, 1953); in a few species of *Limonium* of the Plumbaginaceae (Rao and Das, 1968; Bokhari, 1970), *Bruguiera gymnorrhiza* Bl. of the Rhizophoraceae (Rao and Sharma, 1968); *Scyphiphora hydrophyllacae* Sm. of the Rubiaceae (Rao and Wan, 1969).

In most of the above cases terminal pattern of sclereid distribution is recognised based on observations of cleared leaf surfaces but only in a few cases, that the early development stages have been studied. In some recent ontogenetic studies on leaves of the following terminal sclereid bearing taxa *Mouriria huberi* Aubl (Foster, 1947), *Boronia serrulata* Sm.

Our thanks are due to Dr K. Subramanyam, Director, Botanical Survey of India, for kind encouragement.

(Foster, 1955 b) *Memecylon heyneanum* L, and *M. lushingtonii* Gamble (Rao, 1958), *M. umbellatum* Burm f., *M. capitellatum* L. and *M. lanceolatum* Bl of Melastomataceae (Bhupal, 1971), *Linociera zeylanica* Gamble of the Oleaceae (Bhupal, 1971), *Niebuhria apetala* Dunn. of the Cappardiaceae (Rao, 1951) and *Cynometra cauliflora* of Leguminosae (Rao, 1953) have revealed that sclereid initials are invariably found near the end of the differentiating procambial strand. Thus one could see from the above studies that sclereid initials are intimately, connected with the differentiating procambial strands. The exact morphological or physiological significance of relationship is not yet known.

Another significant pattern worthy of consideration is the form and position of terminal sclereids in the mesophyll. In several investigated species, the lamina sectors have one type of sclereid more or less conforming to one baseform with little modification which do not disturb the body shape. In view of their origin near the procambial strands they have more or less a mid-mesophyll position and the adult sclereids extend all over the mesophyll.

DIFFUSE PATTERN

The diffuse pattern is a wide spread feature in many of the foliar sclereid-bearing taxa of the Phanerogams. In this pattern, sclereids are found to be solitary or grouped dispersed in the mesophyll without any relationship with the vein endings. Their surface distribution in the leaf expanse is variable from species to species. They may be densely set to give a loose tissue-like appearance or disposed widely and evenly in the leaf expanse as exemplified in *Trochodendron aralioides* Sieb et Zuce (Foster, 1945a, b). Recently, this pattern is reported in *Drimys, Belliolum, Bubbia, Zygogynum* and *Illicium* (Bailey and Nast, 1944, 1948), in many species of the Oleaceae and Aroideae (Rao, 1949, 1964; Arjee, 1953a, b).

Ontogenetic studies have shown that diffuse sclereids may arise from any part of the mesophyll. It may also arise from cells in the future palisade as reported in many members of Oleaceae (Rao, 1957: Rao and Kulkarni, 1952; Bhupal, 1971) or spongy cells as seen in *Diospyros discolor* Willd. (Rao 1951b). and *Trochodendron aralioides* Sieb et Zuce. (Foster, 1945a, b) and from cells in both regions as reported in *Olea europaea* L. (Arjee, 1953b). In conclusion, it may be remarked in this connection, in agreement with Foster (1955b), that free sclereid origin in general are unpredictable in their distribution and time of origin in the developing lamina.

MIXED PATTERN

The mixed type of distribution of sclereid is reported only in certain species of the same genus. In *Bornella* (Foster, 1955a) two species exhibit distinct terminal sclereids while in other two species of the same genus both terminal and diffuse sclereids occur in the different leaves of the same collection. The mixed pattern of distribution is also recorded in *Mouriria guianensis* Aubl. (Foster, 1946; Bhupal 1971). *Popowia pisocarpa* (Bl.) Endl. of Annonaceae (Rao and Wee, 1966). The presence of seemingly terminal sclereids in an otherwise diffuse pattern of sclereids recorded in certain species like *Diospyros discolor* Willd (Rao, 1951b). *Ternstroemia japonica* L. (Rao, 1952), *Linociera intermedia* Wight, *L. insignis* C. B. Clarke (Rao, 1957b) have revealed that seemingly terminal sclereids arise away from the procambial strand. They are transformed spongy cell and their eventual terminal relationship is due to the juxtaposed development of the veins ending. The ontogenetic studies in many taxa have revealed that sclereid initials take their origin in palisade region, especially in a few members of the genera *Olea, Linociera* and *Osmanthus* (Arjee, 1953b, Rao, 1949, 1951; Rao and Kulkarni, 1952; Griffith, 1968) whereas in many investigated plants sclereid initials are nothing but transformed spongy cell. In veiw of these findings Rao (1951) has questioned the use of the term 'terminal' for sclereids merely because of their position in the mature lamina. Further, he has emphasised the need for ontogenetic studies before establishing the relationship with tracheary elements.

An interesting feature in sections of the laminae is the presence and distribution of sclereids in the mesophyll. Sclereids are found in the mid-mesophyll region either connected to the vein bundles or away from them. In all such cases it has been shown by several authors that they conform to one base form with slight modifications without much change in their body shape. However, in some cases different types of sclereids are reported to occur in one and the same lamina and often they show various specific combinations coupled with distinctive distribution pattern.

The presence of two types of sclereids in the same leaf is reported in *Memecylon cuneatum* Thw. where columnar and fusiform sclereids form vertical and subtransverse girders respectively (Rao, 1957). In *Exbucklandia populnea* (R. Br. ex. Griff.) R. W. Brown (Syn = *Symingtonia populnea* (R. Br. ex. Griff.) Steen., where idiosclerieds sensu Richter (1920) cluster round the veinlet endings and fusiform sclereids form a strand along the leaf margin (Rao, 1968). Similarly in *Phaenthus pisocarpa* (Bl.) Endl. of the Annonaceae (Rao and Wee 1966). columnar and fibriform sclereids are reported to be present. In a very recent paper, Bokhari and Burtt (1970) have shown in a few species of *Cyrtandra* of Gesneriaceae different types of sclereids confined only in hypodermis or to the mesophyll alone or found both in the hypodermis and also in the mesophyll. Further, they have shown that the resulting feature of their distinctive distribution pattern is very significant in the identification of several species of this genus.

It is of interest to record that origin and sequence of maturation of sclereids have been worked so far, in a very few representative of the angiosperms as a whole. Foster (1955b) was the first to point out that terminal sclereid initials of *Boronia serrulata* Sm. originate in basipetal sequence, their progressive formation coinciding with the basipetal order of maturation of ultimate veinlet in the lamina. While studying the origin and structure of terminal sclereids in *Niebuhria apetala* Dunn. of Capparidaceae Rao (1958) briefly mentions that the sclereid initial originate progressively near the mid-rib, the margin and extend over to the remaining sub-marginal portions of the lamina. Further, he says that the sequence of maturation of veinlet ends and sclereids follow the same pattern. This mode of centripetal and centrifugal pattern of initiation and maturation stands in contrast with that of basipetal sequence of initiation and maturation of sclereids and veinlets. Foard (1958) reports in *Camellia japonica* L. sclereids development in basipetal sequence. Rao and Vaz (1969) working on the foliar sclereids of *Fagraea fragrans* Roxb. report that the first sclereids develop in the midrib region. In view of the description of variable sequential development of foliar sclereids, it is very necessary to work some more examples to determine the serveral mode of development that may exist in many sclereid bearing taxa.

Another interesting and promising field of research is that of experimental side. It is concerned with the determination of sclereid initiation, induction, development and formation in certain species of selereid bearing taxa. Al-Talib and Torrey (1961) have come to the conclusion that sclereid initiation in leaves of *Pseudosuga menziiesii* (Mirb.) Franco. is clearly under some precise control of changing physiological conditions active during leaf and shoot development. Mia and Pathak's (1965) work concerns only the detection of enzymatic activity of cytochrome oxidase in sclereid initials in four species of *Rauvolfia.* Foard's work (1958, 1959) supports the view that the formation and developmental pattern of even highly individualistic cells, such as the idioblastic sclereids of *Camellia japonica* L. are under the control of a higher unit than the cells themselves. It is clear from the above research that the future trend is closely towards the experimental side.

REFERENCES

Al-Talib, K. H., and J. G. Torrey. 1961. Scleried distribution in the leaves of *Pseudotsuga* under natural and experimental conditions. *Am. J. Bot.* **48:** 71-79.

Arjee, Tova. 1953a. Morphology and ontogeny of foliar sclereids in *Olea europaea.* I. Distribution and structure. *Am. J. Bot.* **40:** 680-687.

——. 1953b. Morphology and ontogeny of foliar sclereids in *Olea europaea*. II. Ontogeny. *Am. J. Bot.* **40:** 745-752.

Bailey, I. W., and C. G. Nast. 1944. The compatative morphology of the Wintereceae. V. Foliar epidermis and sclerenchyma. *J. Arnold Arbor.* **25:** 342-348.

——, and——1948. Morphology and relation ship of *Illicium., Schizandra* and *Kadsura.* I stem and leaf. *J. Arnold Arbor.* **29:** 342-348.

Bhupal, C. P. 1971. *Studies on Foliar Sclereids in a Few Taxa of Angiosperms.* Ph. D. *Thesis,* Calcutta University.

Bhupal, O. P., and B. C. Kundu. 1969. Systematics of *Rhodoleia* with reference to sclereid morphology. *Trans. Bose. Res. Inst.* **32:** 31-48.

Bokhari, M. H. 1970. Morphology and taxonomic significance of foliar sclereids in *Limonium. Notes Royal Bot. Gard.* **30:** 43-54.

——. and B. I. Burtt. 1970. Studies in the Gesneriaceae of the old World XXXII. Foliar sclereids in *Cyrtandra. Notes Royal Bot. Gard.* **30:** 11-12.

Foard, D. E. 1958. An experimental study of sclereids development in the leaf of *Camellia japonica. Pl. Physiol.* **33** (suppl.): 12.

——. 1959. Pattern and control of sclereid formation of *Camellia japonica. Nature, Lond.* **184:** 1663-1664.

Foster, A. S. 1945a. The foliar sclereids of *Trochodendron aralioides* Sieb. and Zucc. *J. Arnold Arbor.* **26:** 155-162.

——. 1945b. Origin and development of sclereids in the foliage leaf of *Trochodendron aralioides* Sieb. and Zucc. *Am. J. Bot.* **32:** 456-485.

——. 1946. Comparative morphology of the foliar sclereids in the genus *Mouriria* Aubl. *J. Arnold Arbor.* **27-** 253-271.

——. 1947. Structure and ontogeny of the terminal sclereids in the leaf of *Mouriria huberi* Cong. *Am. J. Bot.* **34:** 501-514.

——. 1955a. Comparative morphology of the foliar sclereids in *Boronella* Baill. *J. Arnold Arbor.* **36:** 189-198.

——. 1955b. Structure and ontogeny of terminal sclereids in *Boronia serrulata. Am. J. Bot.* **42:** 551-560.

Foster, A. S. 1956. Plant idioblasts: remarkable examples of cell specialisation. *Protoplasma* **26:** 184-193.

Griffith, M. M. 1968. The structure and development of foliar sclereids in *Osmanthus fragrans* L. *Phytomorphology* **18:** 75-79.

Mia, Abdul J., and S. M. Pathak. 1965. Histochemical studies of sclereids induction in the shoot of *Rauwolfia species I.* Cytochrome oxide. *J. exp. Bot.* **16:** (46). 177.

Morley, T. 1953a. The genus *Mouriri* (Melastomataceae) a sectional revision based on anatomy and morphology. *Univ. Calif. Publs Bot.* **26:** 223-312.

——. 1953b. A new genus and three new species in the *Memecyleae* (Melastomataceae). *Am. J. Bot.* **40:** 248-255.

Rao, A. N., and Wee Yeo Chin. 1966. Foliar sclereids in certain members of Annonaceae and Myristicaceae. *Flora* B **156:** 220-231.

——, and L. C. Wan. 1969. A new record of foliar sclereids in *Scyphiphora hydrophyllaceae Curr. Sci.* **38:** 594-595.

——, and S. J. Vaz. 1969. Morphogenesis of foliarsclereids. I Ontogeny and distribution of foliar sclereids in *Fagraea fragrans. Phytomorphology* **19:** 159-169.

Rao, A. R., and M. Sharma. 1968. The terminal sclereids and tracheids of *Bruguiera gymnorrhiza* Bl. and the cauline sclereids of *Ceriops roxburghiana* Ar. *Proc. natn. Inst. Sci. India.* **34:** 267-275.

Rao, T. A. 1949. Foliar sclereids in the oleaceae. I. On the occurrence of the sac like spicular cells in the leaf of *Schrebera swietennoides* Roxb. *J. Indian bot. Soc.* **28:** 251-254.

——. 1951a. Studies on foliar sclereids. A preliminary survey. *J. Indian bot. Soc.* **30:** 28-39.

——. 1951b. Studies on foliar sclereids in Dicotyledons. I. Structure and ontogeny of sclereids in the leaf of *Diospyros discolor* Willd. *Proc. Indian Acad. Sci.* **B34:** 92-98.

——. 1952. Studies on foliar sclereids in Dicotyledons. IV. Structure and development of sclereids in the leaf of *Ternstroemia japonica* L. *Proc. natn. Inst. Sci. India.* **B18:** 233-239.

——. 1953. *Studies on Foliar Sclereids in Seed Plants.* Ph. D. *Thesis.* Mysore University, Mysore.

——. 1957a. Comparative morphology and ontogeny of foliar sclereids in seed plants. I. *Memecylon* L. *Phytomorphology* **7:** 306-330.

——. 1957b. Comparative morphology and ontogeny of foliar sclereids in seed plants. II. *Linociera* Swartz. *Proc. natn. Inst. Sci. India.* **B23:** 152-164.

——. 1958. Studies on foliar sclereids in Dicotyledons. VI. Origin and development of terminal sclereids in the leaf of *Niebuhria apetala* Dunn. *Proc. Indian Acad. Sci.* **B47:** 223-228.

——. 1964. On the Multi-septate Trichosclereids in the genus *Scindapsus*. *Proc. Indian Acad. Sci.* **B60:** 66-69.

——. 1968. Distributional pattern of sclereids in the leaf and stipules of *Symingtonia populnea* (R. Br. ex Griff.) Steen. (Hamamelidaceae). *Curr. Sci.* **37:** 352-354.

——, and G. C. Das. 1968. Foliar sclereids in some species of *Limonium* (Plumbaginaceae). *Curr. Sci.* **37:** 352-354.

——, and G. Y. Kulkarny. 1952. Foliar sclereids in the Oleaceae. III. Ontogeny of the sclereids in two species of the genus *Olea* L. *Jour. Univ. Bombay* **B20:** 52-57.

——, and K. J. Mody. 1961. On terminal sclereids and tracheoid idioblasts. *Proc. Indian Acad. Sci.* **B53:** 267-262.

Richter, A. 1920. Über einige neue Glie der Marcgraviaceen, auf Basis der phylogenic und der vergleichenie Anatomic. *Math. Naturw. Ber. Ung.* **31:** 65-146.

Schulze, H. 1902. Beiträge zur Blatt enanatomic der Rutaceen. *Beih. bot. Zbl.* **12:** 55-98.

18435

Adv. Pl. Morph. 1972: 51–57

MINOR VENATION PATTERN IN THE INDIAN ACERS

BY M. L. BANERJI AND SITESH DAS
Kalyani University, Kalyani, India

ABSTRACT

Some recent studies have emphasized the taxonomic importance of venation and its usefulness in distinguishing genera and species in some cases. The authors have devoted some attention to the minor veins during the study of the Indian species of *Acer*. The pattern of major venation in the leaves of the majority of species is craspedodromous while in some species e.g. *Acer oblongum, A. niveum* and *A. laevigatum* the pattern is acrodromous. In some species there are free terminations of the veinlets while in others the eyelets lack free vein-endings. The largest number of species of the Indian Acers fall under the first type. The size of the areoles, number of terminations per areoles, coupled with other characteristics such as the number of branches, character of branches, whether stout or slender and the 'very tips' of the branches provide useful characters for the identification besides taking consideration the number of basal nerves. Howard (1962) views that the vascular structure of the petiole appears to be the most useful at generic level, and thus the description of the vascular pattern of the petiole is an additional tool to aid the horticultural taxonomist in the study. In the opinion of the authors, the minor venation patterns provide useful taxonomic clues in the genus *Acer* as well for the identification of sterile plant material.

INTRODUCTION

The classical work of Ettinghausen (1861) though fundamental and still unsurpassed in kind, now over 100 years old, leaves much to be desired. The vast majority of the dicot leaves exhibit a wide range of venation patterns, both of the major and of the minor veins, which is generally complex. Foster (1950a) has expressed the need for extensive and intensive exploration of venation pattern in the leaves of angiosperms, and in recent years the study of minor venation patterns has been receiving considerable attention as it is felt that use of major and minor venation in taxonomic applications is in its infancy. However, some of the the recent studies have emphasized the taxonomic importance of venation and its usefulness in distinguishing genera and species in some cases. The authors have devoted their attention to the minor veins during the study of the Indian species of *Acer*.

MATERIAL AND METHODS

Leaves or portions of leaves of all the Indian species of *Acer* were obtained from herbarium specimens. The material was secured from Central National Herbarium, Calcutta, and Herbarium (Department of Medicinal Plants, Kathmandu, Nepal) and amply supplemented by herbarium material collected by the senior author. Because of the number of herbarium sheets utilised is so large, a complete list is not possible. However, reference to sheets is given in the microphotographs.

Portions of leaves were either simply boiled in water or else soaked for 24 hours in water, later soaked for over night in 2.5% ammonium hydroxide, washed and stained in alcoholic solution of Safranin. But better results were obtained when leaf-segments were cleared in 5% NaOH and then in Chloral hydrate, following Arnott (1959). Cleared materials were stained in alcoholic solution of Safranin, dehydrated and mounted in Canada balsam. Stained segments of leaf mounted in glycerine jelly have given better photographs. Portions from different parts of the lamina have been examined and it has been found that there is regularity of the minor veins in the lower parts of the lamina. Thus, study has been made of the lower 2/3rd of the lamina of a mature leaf. However, the apical 1/3rd of the lamina has not been ignored altogether. Depending on the availability of very young and of leaves of differing maturity, study has been made of such materials.

OBSERVATIONS

Leaves of the Indian species of *Acer* are always simple, but the lamina may be lobed, which are in some species 3, in others 5 and in still others as many as 7. Similarly the number of basal nerves is also variable in the different species—it may be 3, 5, or 7. This character of the number of basal nerves has been freely used by Hiern (1875) in the key to the species of *Acer* in Hooker's Flora British India. In an attempt to have additional characteristics for comparison, leaves have been studied as regards the minor venation patterns.

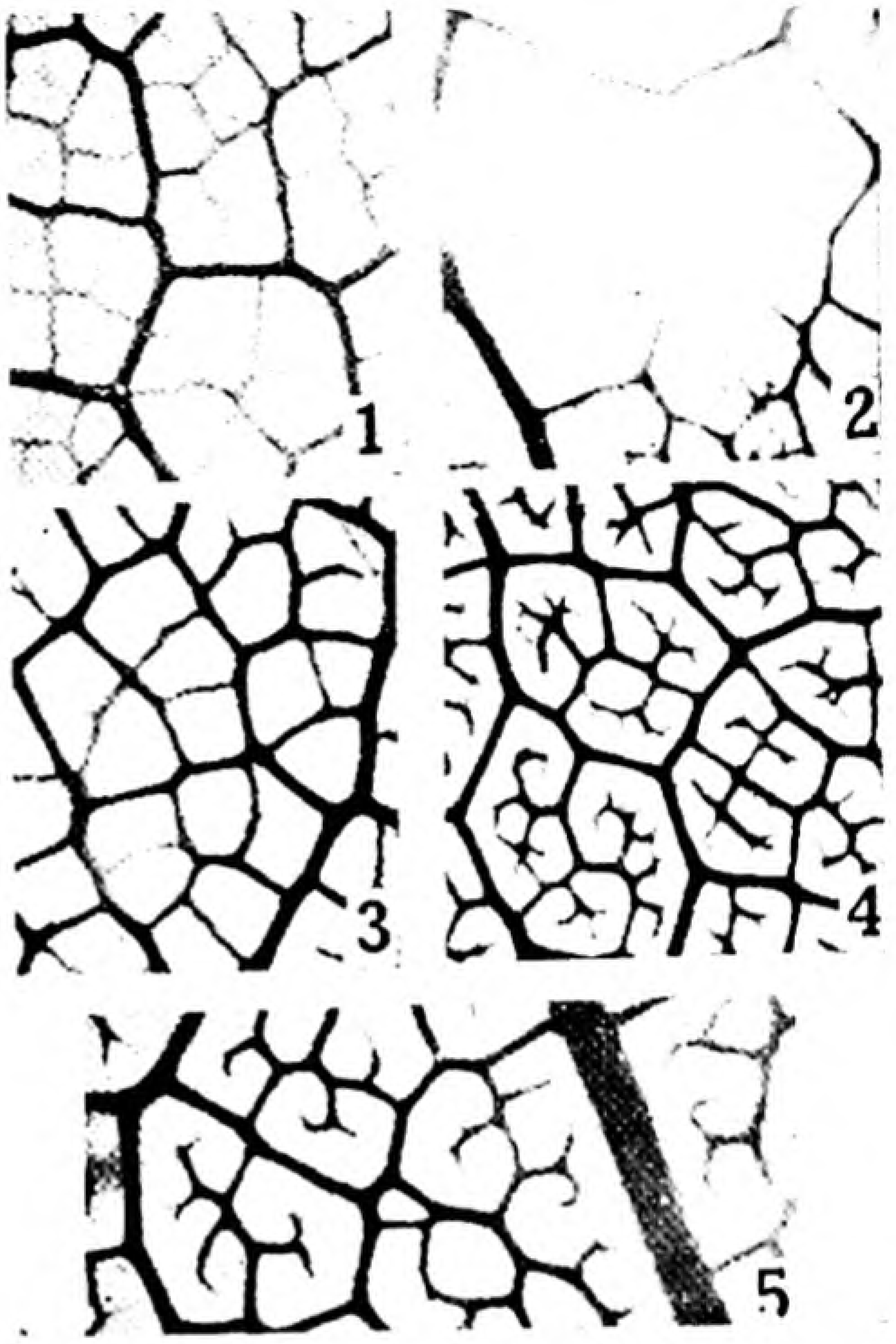
The pattern of major venation in the leaves of the majority of species is craspedodromous (Ettinghausen, 1861), i.e. the secondary veins follow a direct course towards the leaf margin and terminate. In *A. oblongum* Wall., *A. niveum* Bl. and *A. laevigatum* Wall., species with undivided leaves, the pattern is acrodromous. The veins of the first, second and third category are considered to constitute the major venation of leaves (Pray, 1954). Veins of the fourth, fifth and sixth categories and the vein-endings (ultimate veinlets) which terminate freely in the mesophyll of some of the species, constitute the minor venation that form the reticulum. In some species there are free terminations of the veinlets, while in others the eyelets lack free vein-endings. The largest number of species of the Indian *Acers* fall under the first type. The size of the areoles, number of terminations per areoles, coupled with other characteristics such as the number of branches, character of branches, whether stout or slender and the 'very tips' of the branches provide useful characters for the identification besides taking consideration the number of basal nerves.

In species which have free terminations, the vein-endings generally branch 1-3 times dichotomously. In species which lack free terminations, the veinlets of juvenile leaves branch further and give rise to branches of the next category, which finally anastomose amongst themselves or with veins of higher categories resulting in the formation of 'ultimate areoles'. These 'ultimate areoles' or areas of mesophyll that are clearly circumscribed, are irregularly rectangular or polygonal. Bundle sheath is present in all the species studied, and the sheath extends over the veinlets of all categories except the last ones, and the sheath is composed of parenchyma.

On a careful study of a large number of leaves of *A. oblongum* Wall. and *A. laevigatum* Wall., it is our observation that there are infact 5 basal nerves in *oblongum* and not 3 as mentioned by Hiern (loc. cit.) while in *A. laevigatum* there are only 3 basal nerves. In *A. oblongum* the situation being that there is a prominent mid-nerve on either side of which there is another nerve but less prominent, and finally is a nerve on each side close to the margin on the lamina which is the least prominent. There are significant differences in the minor venation pattern of the two species. In *A. oblongum* the areoles do not have free terminations of the veinlets (fig. 1) whereas in *A. laevigatum* there are free termination of the branches of the veinlets of the last category (Fig. 2).

Externally the leaves of *A. campbellii* Hk. f. & Th. ex Hiern and *A. cappadoicicum* Gledt. have a resemblance as regards the number of lobes and the number of basal nerves. There are also other differences such as the shape of the lamina and its margin. However, the minor venation pattern is strikingly different, not only in the two species but also between the two varieties of *A. campbellii*. In *A. cappadoicicum* the areoles are without free terminations of the veinlets (Fig. 3), while in *campbellii* the areoles are with free terminations. There exist differences between the two vars. of *A. campbellii*; in var. *campbellii* the ultimate veins are more dense (Fig. 4) as compared to var. *serratifolia* (Fig. 5).

Leaves of *A. sikkimense* Miq. closely resemble those of *A. hookeri* Miq. except for the minutely serrate margin of the former and the duplicato-serration of the later. *Pax* (1902) has mentioned two varieties of *A. sikkimense* depending on the serrulation of the leaves. A study of the minor venation pattern and the areoles has shown more differences between the two species. As observed, the free terminations of the veins are much slender and the veins of lower category are usually dichotomously branched in *A. hookeri* (Fig. 6), while on the other hand the areoles of *A. sikkimense* have thick and stout vein endings (Fig. 7). On this characteristic feature of the ultimate veinlets, we are strongly of the opinion

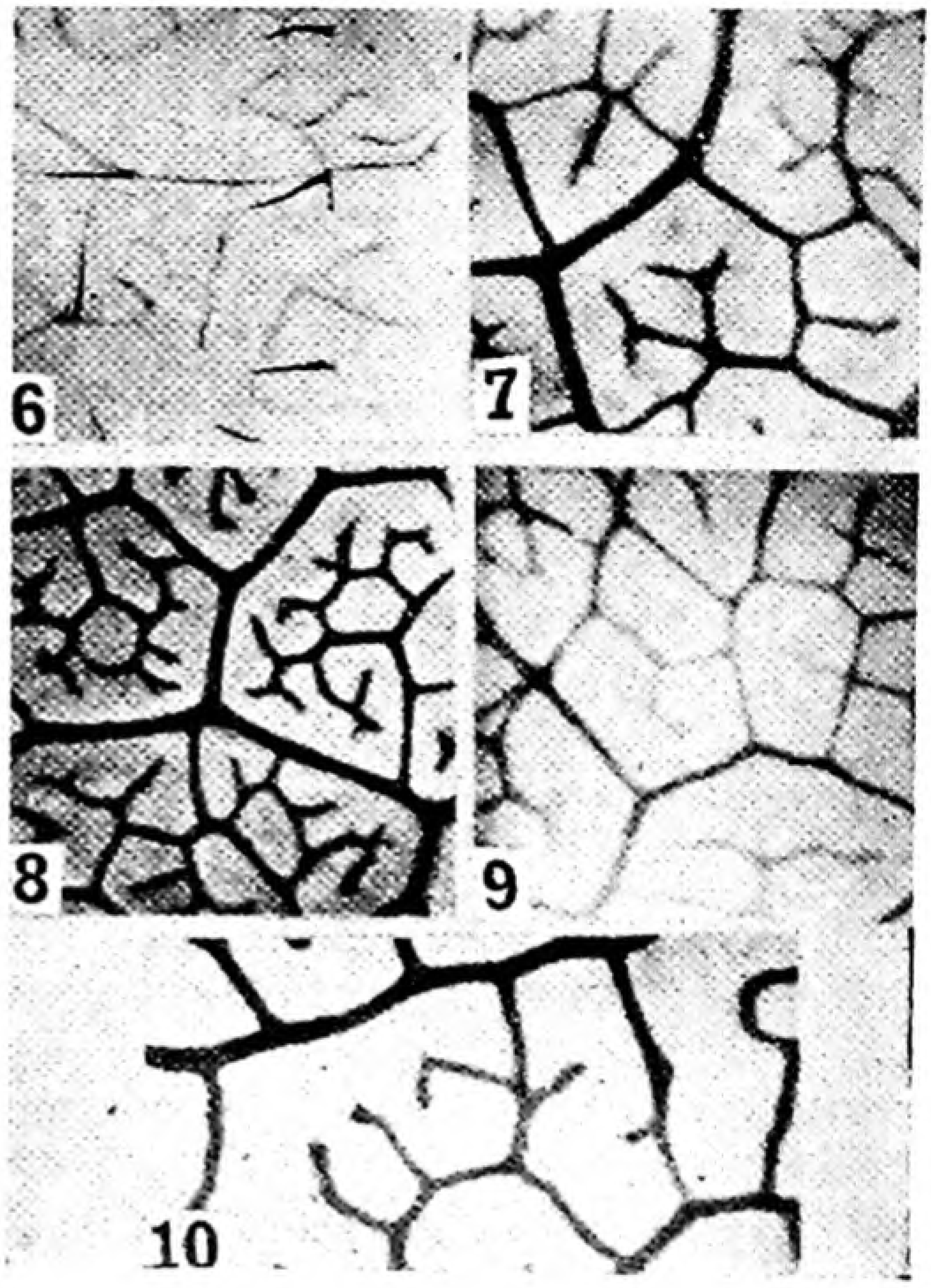


Figs. 1-5. Veinlet endings in cleared leaves of *Acer* spp. Fig. 1. Venation pattern of a cleared. *A. oblongum* Wall. leaf. (B. sn. ex. herb. Banerji). Fig. 2 Venation pattern of a cleared *A. laevigatum* Wall. leaf (Mackinnon, sn. ex herb. Calcutta). Fig. 3. Venation pattern of a cleared. *A. cappadoicicum* Gledt. leaf (Nepal Herb, no. 4167). Fig. 4. Venation pattern of a cleared *A. campbellii* Hk. f. & Th. ex Hiern, var. *campbellii* leaf (B. 774 ex herb. Banerji). Fig. 5. Venation pattern of a cleared *A. campbellii* var. *serratifolia* leaf (S. S. W. 5413 ex herb. Banerji).

that Griffith 936 which has been mentioned by Pax as belonging to *A. sikkimense* is not correct; it is *A. hookeri*. It is pertinent to mention that Griffith 936 is a sterile material, and if the fruits had been present, then there would have been no dispute as regards the correct identity of the specimen, because the fruits are characteristically different in the two species.

A. pectinatum Wall. was treated as a *forma* of *A. caudatum* Wall. by Hiern (1875); he also treated *A. acuminatum* Wall. ex. D. Don, as a synonym of *A. caudatum*. But Pax (1902) has treated it as a distinct species. And *A. papilio*, a species discribed by King, has not been accepted by Pax (loc. cit.) who has treated it as synonymous to *A. acuminatum* with which it is very often confused. A study of the minor veins has brought to light the differences and clarifies the confusion. The areoles of *A. acuminatum* have free vein-endings which are stout, while in *A. pectinatum* the vein endings are slender (Fig. 9). Although the vein endings are stout in both *A. acuminatum* and *A. papilio*, the vein spacing in *A. papilio* is very little as compared with *acuminatum*. Further, the veinlets of *A. acuminatum* anatomose among themselves to form loop-shaped 'ultimate areoles'. [illegible] situation occurs but ra[illegible] there appears to be a tend[illegible] very tips' of branches [illegible] horizontal spreading [illegible] loop-shaped 'ultimate areoles' [illegible] also rarely seen in *A. s*[illegible]

According to [illegible] view that signific[illegible] vein eyelets [illegible] of the plant, has b[illegible] foundation. Our st[illegible] species that occur in In[illegible] of *A. sterculiaceum*, *A.* [illegible] *A. laevigatum* show that with increasing age of the leaves, there are significant changes not only in the size but also in the character of the eyelets. For example,

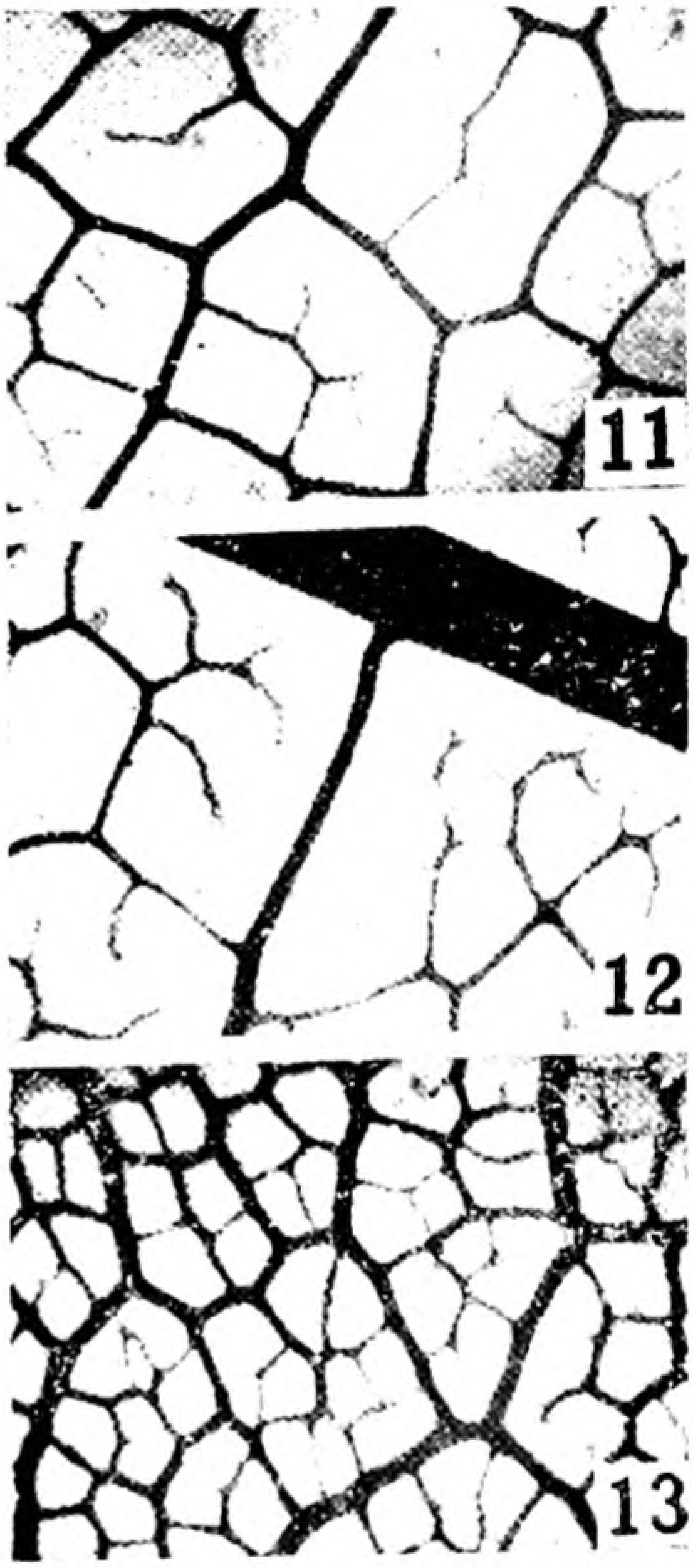


FIGS. 6-10. Veinlet endings in cleared leaves of *Acer* spp. Fig. 6. Venation pattern of a cleared *A. hookeri* Miq. leaf (Osmaston sn. ex herb. Calcutta.). Fig. 7. Venation pattern of a cleared *A. sikkemense* Miq. leaf. (Nepal herb. no. 5717). Fig. 8. Venation pattern of a cleared *A. acuminatum* leaf (P. S. W. 3782 ex. Nepal herb.). Fig. 9. Venation pattern of a cleared *A. pectinatum* Wall. leaf (JDS. 297 ex Nepal herb.). Fig. 10. Venation pattern of a cleared *A. papilio* King. leaf (B. 1060 ex herb. Banerji).

[illegible] *sterculiaceum* the very young leaf [illegible] areoles which have free terminations [illegible] with increase in age the veinlets [illegible] to branch and these vein branches [illegible] stout, finally these branches [illegible] with other branches of the [illegible] category or even with branches [illegible] category, resulting in the formation of 'ultimate areoles' and the resulting areoles have few or even no free endings of veinlets. Such anastomosis of veinlets has also been in *A. laevigatum*, but it is of rare occurrence. It is the gradual differentiation of tracheids and their anastomosis by slight elongation that results in the formation of these 'ultimate areoles'. In case of *A. oblongum* the observations have been also in the same direction. The young leaves have areoles without free endings of veinlets, and when the leaf matures, still the areoles remain without free terminations, but the veinlets and veins become stout. In the young leaves of *A. laevigatum* the areoles have free terminations of the veins and as the leaves advance in age, the minor veins branch further and become stout. Thus, in a single areole of a mature leaf there are more vein terminations as compared to the areole of a young immature leaf. This shows that in *Acer* there are significant changes in the veinlets and also in the areoles with increasing age of the leaf. It is to be noted that according to Foster (1950 a) there is 'progressive increase in complexity of the venation pattern' in Quiinaceae. A similar observation has been of Tucker (1964) in the leaves of different maturity of *Magnolia tripetala*. Kasapligil (1964) has also studied the anatomical differnces in the minor venation of juvenile leaves and the adult leaves of *Corylus*. All this shows that in some cases there are significant changes in the venation pattern of the minor veins with increase of age of the leaf.

DISCUSSION

According to Kasapligil (1964) in *Corylus avellana* and *C. maxima* the ultimate areoles may or may not be provided with a vein ending, while in *C. colurna* most of them are provided with freely terminating veins. Our observation on *Acer oblongum* and *A. laevigatum* also show such interspecific differences. As regards vein spacing, what we observe in *A. campbellii* v. *campbellii* and v. *serratifolia*, very similar differences have been reported by Hardin (1964, 1968) in *Phytolacca americana* and *P.*



FIGS. 11-13. Veinlet endings in cleared leaves of *Acer* spp. Fig. 11. Venation pattern of a cleared *A. sterculiaceum* Wall. young leaf (S.S.W. 4945 ex Nepal herb.). Fig. 12. Venation pattern of a cleared *A. sterculiaceum* Wall. semi mature leaf (Nepal herb no. 4080). Fig. 13. Venation of a cleared *A. sterculiaceum* Wall. fully mature leaf (B.U.B. 3432 ex herb. Banerji).

rigidea and also in *Diervilla lonicera, D. sessilifolia* and *D. rivularis*. Handro (1967) also emphasises on the vein spacing differences in *Pfaffia sericea, P. jubata, P. gnaphalioides* and *P. velutina*. That the 'very tips' of the ultimate veins branch and expand has been reported by Kasapligil (loc. cit.) in *Corylus colurna* where loop-shaped areoles are formed. Loop-shaped 'ultimate areoles' have been found by us to occur in *Acer acuminatum*.

Although venation patterns distinct from those commonly known to occur in dicots have been found in *Quiinaceae*, the vast majority of the dicot leaves exhibit a wide range of patterns. View is expressed by Howard (1962) as regards the vascular structure of the petiole of certain families e.g., Leguminoseae Fagaceae, Rosaceae, Araliaceae and Icacinaceae where the petiole vascular pattern can be profitably used in description and determinations. Howard also views that the vascular structure of the petiole appears to be the most useful at generic level, and thus the description of the vascular pattern of the petiole is an additional tool to aid the horticultural taxonomist in the study and determination of leafy plant material. In a series of papers on the family Quiinaceae, Foster (195[illegible]) has shown that careful description of venation together with other details of anatomy can yield valuable [illegible] dividends. Also, according to Carlquist's (1959) minor venation provides taxonomic and evolutionary clues in certain Hawaiian compositae. Handro (1964, 1967) demonstrates the possibility of identification of plants of the family Amaranthaceae through the use of vegetative characters at the generic and also at the specific level. It may be added that Dede (1962) distinguishes a series of venation types in the Rutaceae, and a key based on the characteristics of the cavities and their associated venation in the leaf is constructed by him. The usefulness of veinlet endings as a diagnostic feature has been illustrated by a comparison of two species of *Liriodendron—L. chinense* and *L. tulipifera* by Tucker (1964). There exist inter-specific differences in the venation pattern in *Trifolium* also (personal communication from Prof. Foster). In the opinion of the authors, the minor venation patterns provide useful taxonomic clues in the genus *Acer* as well for the identification of sterile plant material.

Although Nicely (1965) found great variation in the same leaf as regards the size and shape of vein islets and the number of vein endings in each, such has not been mentioned by any of the other workers. However, we have found that near the apical portion of the lamina there are indeed some variations and as such portions of the lamina which are 2/3rd from the base of a fully mature leaf should best be studied.

REFERENCES

ARNOTT, H. J. 1959. Leaf [illegible] *News* 37: 192-194.

CARLQUIST, S. 1959. [illegible] *Dubautia*, [illegible] positae) *Pacif. Sci.* 13: [illegible].

DEDE, ROLAND A. 1962. [illegible] in the Rutaceae. *Ann.* [illegible]

EAMES, A. J. 1961. *Morphology of the Angiosperms* McGraw-Hill, London.

ETTINGHAUSE, C. 1861. Die Blatt-Skelete der Dicotyledonen mit besonderer Rucksitcht auf die Untersuchung und Bestimung der fossilen Pflanzenreste. K. K. Hofund-staats-druckerie. Wien.

---

* not consulted in original.

FOSTER, A. S. 1950a. Morphology and venation of the leaf of *Quiinia acutangula* Ducke. *Am. J. Bot.* **37:** 159-171.

——. 1950b. Venation and history of the leaflets in *Touroulia guianensis* Aubl. and *Froesia tricarpa* Piers. *Am. J. Bot.* **37:** 848-862.

——. 1951. Heterophylly and foliar venation in *Lacunaria. Bull. Torrey bot. Club.* **78:** 382-400.

HANDRO, WALTTER. 1964. Contribuicao ao estudo da venacao e anatomia foliar das Amarantaceas dos Cerrados I. *Annls. Acad. barasil. Cienc.* **36:** 479-499.

——. 1967. Contribucao ao estudo da venacao e anatomia foliar das Amarantaceas dos Cerrados II. *Annls. Acad. barasil Cienc.* **39:** 495-506.

HARDIN, JAMES W. 1964. A comparison of *Phytolacca americana* and *P. rigida. Castanea.* **29:** 155-164.

——. 1968. *Diervilla* (Caprifoliaceae) of the South eastern U. S. *Castanea* **33:** 31-36.

HIERN, W. P. 1875. In J. D. Hooker's *Flora British India.* **1:** Reeves, London. 692-696.

HOWARD, R. A. 1962. The vascular structure of the petiole as a taxonomic character. *Adv. in Hort. Sci.* Pergamon Press. N. Y. **3:** 7-13.

KASAPLIGIL, BAKI. 1964. A contribution to the histotaxonomy of *Corylus* (Betulaceae). *Adansonio* (*N.S.*) **4:** 43-90.

NICELY, KENNITH, A. 1965. A monographic study of the Calycanthaceae. *Castanea.* **30:** 38-81.

PAX, F. 1902. In Engler's *das Pflanzenreich.* 8. (rept. 1959)

PRAY, T. R. 1954. Foliar venation of Angiosperms I. Mature venation of *Liriodendron. Am. J. Bot.* **41:** 663-670.

TUCKER, SHIRLEY C. 1964. The terminal idioblasts in magnoliaceous leaves. *Am. J. Bot.* **51:** 1051-1062.

Adv. Pl. Morph. 1972: 58–64

PERIDERM IN SOME DEVONIAN PLANTS

By Stephen E. Scheckler and Harlan P. Banks

Division of Biological Sciences, Cornell University, Ithaca, N. Y., U. S. A.

Abstract

The oldest occurrence of periderm in the fossil record is reported in a progymnosperm, *Triloboxylon hallii* (Arnold) Scheckler and Banks 1971. *T. hallii* occurs in Mid-Givetian strata in the Devonian of western New York at approximately 360 million years ago. Periderm is also reported in other progymnosperms in lower Upper Devonian (Frasnian) strata (*T. ashlandicum*, *Tetraxylopteris schmidtii*, and *Proteokalon petryii*). All four species belong to the order Aneurophytales of Progymnospermopsida.

Introduction

One of the by-products of the study of early land plants is the discovery of time of origin of various biocharacters, characters that were assembled gradually into organisms that fit present-day concepts of the several plant groups. When first evolved, some of these characters may have appeared in organisms whose totality of characters fits no modern group of organisms. Such a group is the Progymnospermopsida (Beck, 1960, 1964; Banks, 1968). These plants, found in Middle and Upper Devonian strata, anatomically have much in common with gymnosperms yet they were free-sporing plants. Some, with attached sporangia, are heterosporous. Therefore they are like no group of living plants and required erection of a special category. This category—progymnosperms—is useful for plants with some pteridophytic and some gymnospermous features.

The landscape in New York in Mid and and Late Devonian time was richly covered by plants belonging to Progymnospermopsida. Many of them were preserved as fossils. Some are casts, some compressions, some are petrifactions and some are partially pyritized compressions. Petrifactions by calcium carbonate have been studied in detail for the genus *Callixylon* (Arnold, 1930). But more frequent are axes preserved by iron sulfide (pyrite or marcasite). As a result we have been working for a number of years on pyritized progymnosperms (*Tetraxylopteris* Beck, 1957; Bonamo and Banks, 1967; Matten and Banks, 1967; Scheckler and Banks, 1971a; *Triloboxylon* Matten and Banks, 1966; Scheckler and Banks, 1971a; *Proteokalon* Scheckler and Banks, 1971b; *Archaeopteris* Carluccio, Hueber and Banks, 1966). It is perhaps no surprise that we have found in this group what we believe to be the earliest occurrence of periderm in a fossil plant.

This research was supported in part by National Science Foundation Grants GB 4493 and 8282X, in part by Federal Hatch Funds. We acknowledge with thanks the skilful assistance of Mrs. Jane Blanchard with the photographic work.

Triloboxylon hallii (Arnold) Scheckler and Banks (1971) from the Ludlowville Formation of New York (Table 1) is apparently the oldest plant in which periderm has yet been found. It lived about 360 million years ago. The same tissue also occurs in the slightly younger plants *Triloboxylon ashlandicum, Tetraxylopteris schmidtii* and *Proteokalon petryi*, all of which occur in lower Upper Devonian strata (Table 1—Genesee Group). All four species belong to that group of progymnosperms called Aneurophytales (Scheckler and Banks, 1971a). In addition we are currently studying periderm found in a root that is identified tentatively as an aneurophytalean. Its age is also Upper Devonian. That periderm will ultimately be found in still older, perhaps Eifelian, plants, we have no doubt. Only the future can confirm this supposition.

TABLE I

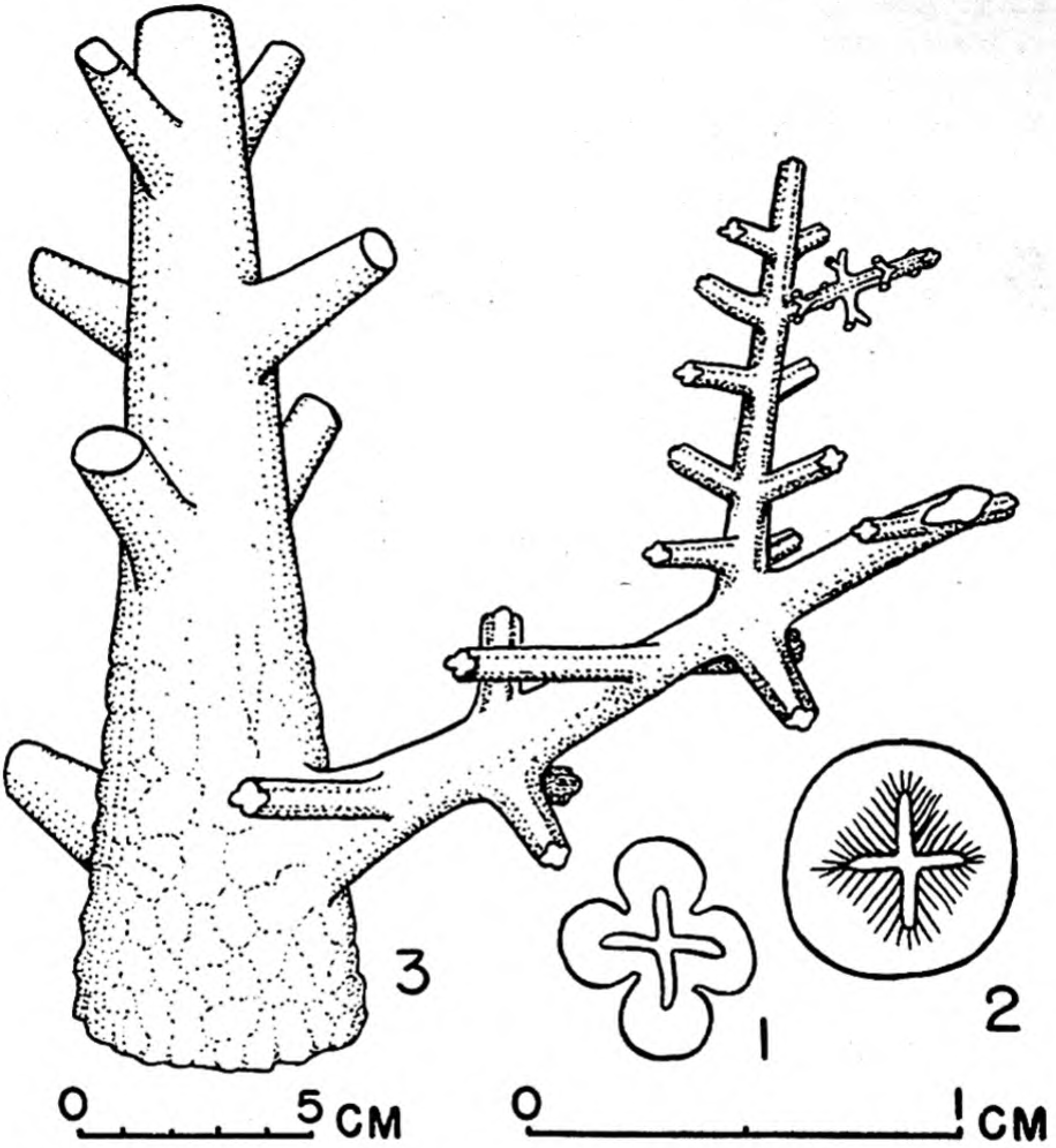
ABBREVIATED SUBDIVISIONS OF MIDDLE AND UPPER DEVONIAN STRATA TO SHOW POSITION OF PERIDERM-BEARING PROGYMNOSPERMS.

Period	Europe	Series	Stage	Group or Formation
UPPER DEVONIAN	Famennian 353	Chautauquan	Bradford	Conewango gp.
			Cassadaga	Conneaut gp. Canadaway gp.
	Frasnian 359	Senecan	Cohocton	Java gp. West Falls gp.
			Finger Lakes	Sonyea gp. Genesee gp.*
			Taghanic	Tully fm.
MIDDLE DEVONIAN	Givetian 364	Erian	Tioughnioga	Moscow fm. Ludlowville fm.*
			Cazenovia	Skaneateles fm. Marcellus fm.
	Eifelian 370		Southwood	Onondaga fm.

* Indicates strata from which they have been collected. Numbers give the time in millions of years ago that the subdivision began.

DESCRIPTION

Young axes of *Triloboxylon, Proteokalon* and *Tetraxylopteris* are deeply three or four-lobed (Fig. 1). Immediately under the epidermis is a fibrous hypodermal outer cortex, then parenchymatous inner cortex, primary phloem and primary xylem. As secondary xylem is produced the outline of the axis gradually becomes terete (Fig. 2). Periderm is initiated relatively late. Our specimens show several stages in development of periderm and show also two increments of growth in the secondary xylem. These growth layers may not represent annual accumulations, but at least the axes are known to be mature and may be beyond the first season of growth.



FIGS. 1-3. *Tetraxylopteris schmidtii.* Fig. 1. Transverse section of young branch to show lobing. Cruciform strand in centre is primary xylem. Fig. 2. Transverse section of older, terete branch. Secondary xylem indicated by radiating lines. Fig. 3. Reconstruction of branching pattern of plant. The marked tapering of the main axis and its roughened surface are the result of periderm formation.

Periderm is initiated (Figs. 5, 6) by periclinal divisions of parenchyma cells located either just inside the fibrous outer cortex or deeper in the inner cortex. It is initiated nearly simultaneously around the whole circumference of the axis. Cells that we interpret as phellogen (Figs. 6, 7, arrow) are thin-walled, radially flattened and rectangular when viewed in either transverse or radial section. Their three-dimensional shape is a tabulate polygon.

Phellem cells are arranged in radial rows without intercellular spaces (Fig. 7). In some specimens the cork cells are thin-walled and the cortical cells outside them are still intact. This may be due to a paucity of wax in the walls of the cork cells and a consequent continuity of water transport to the cortical cells. In other specimens (Fig. 8) the phellem cells are thick-walled. These thick walls lack pits and the wall is lamellated. This may be the result of deposition of alternating layers of suberin and wax as in some extant plants (Esau 1965, p. 340). The thickness of the walls of these phellem cells is increased markedly as they age or as one studies a series of sections cut successively more proximally.

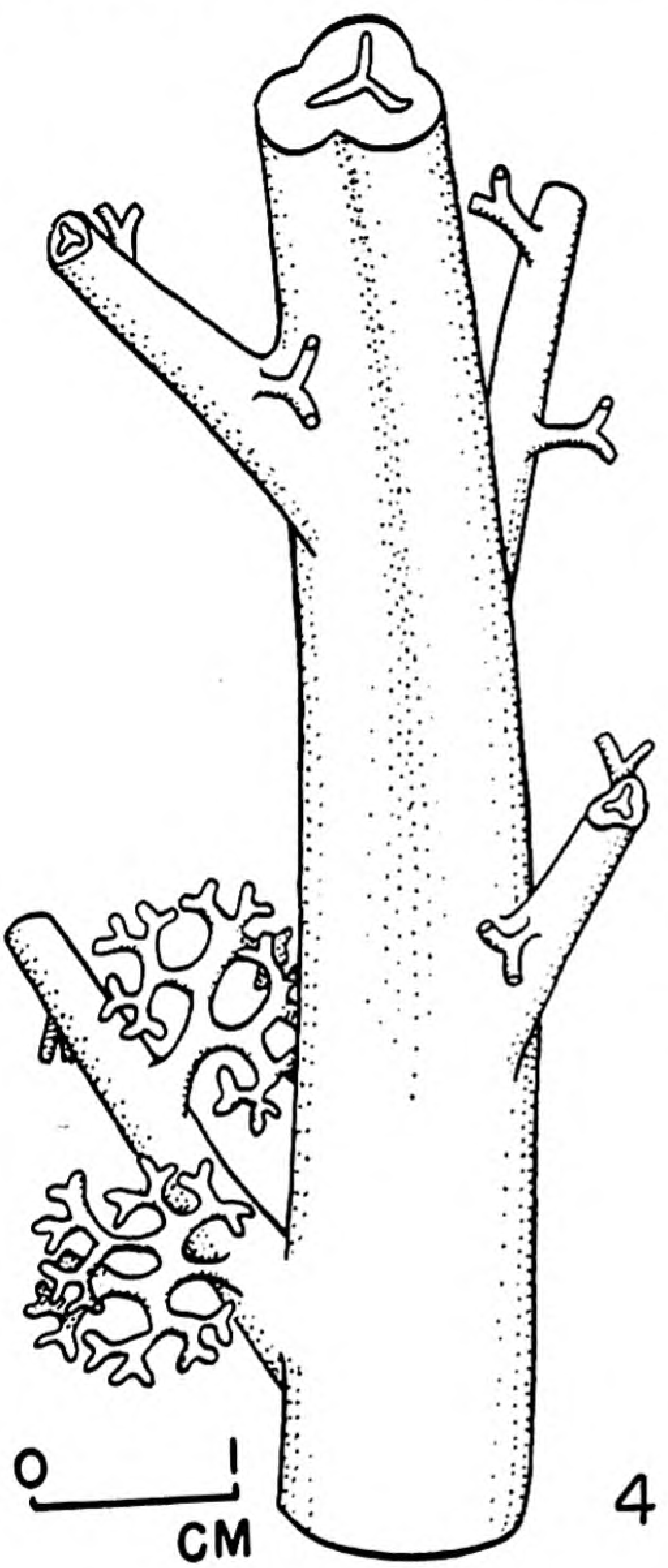


Fig. 4. *Triloboxylon ashlandicum*. Reconstruction of branching of the plant as now known. Ultimate appendages (leaves) shown as repeatedly dichotomized, planated organs.

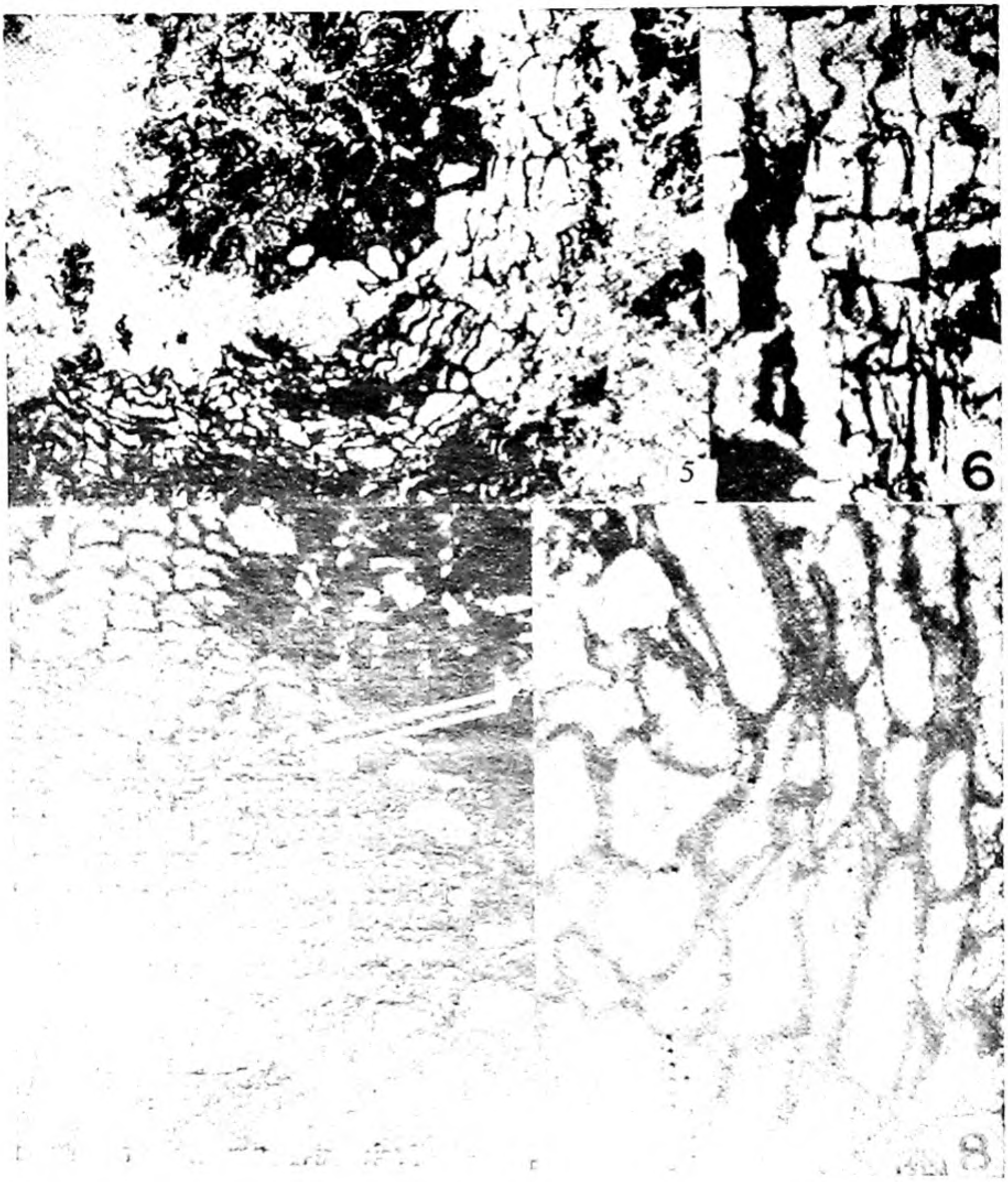
Phelloderm consists of parenchymatous cells similar to cortical parenchyma. However, they are distinguishable by their radial seriation. The phelloderm is surprisingly extensive (Fig. 7) frequently equalling the amount of phellem in our most mature axes. This is not common in extant plants (Fahn, 1967, p. 333).

Once periderm formation has been initiated it is produced rapidly. The resulting increase in diameter produces a marked taper from the older to the younger parts of an axis. This tapering is illustrated for *Tetraxylopteris* in Fig. 3. Eventually the outer primary tissues are sloughed off in such a way that a rough surface results. The phellogen in some axes divides unevenly, producing a larger number of derivatives in some localized areas. In these more active areas the thicker periderm tends to split. In other axes the periderm is more uniform in thickness and the cortex sloughs off irregularly rather than splitting.

DISCUSSION

Periderm usually accompanies the development of secondary vascular tissue (Fahn, 1967, p. 331). It is found in gymnosperms, most dictotyledons, woody lycopods and sphenopsids and in *Botrychium* (Bierhorst, 1971, p. 132). On the basis of other anatomical and morphological features the progymnosperms are regarded by some as ancestral stock whence Paleozoic gymnosperms might have arisen (Beck 1960, 1966; Scheckler and Banks 1971a). The presence of a well-developed periderm provides yet another feature linking the progymnosperms to the gymnosperms.

Filicales lack periderm. Ophioglossales, as represented by *Botrychium,* do produce



[illegible]
oblique [illegible]
cortex to [illegible] early divisions [illegible]
section of periderm [illegible]
cells below. Holotype, [illegible]
phellem cells [illegible]
CUPC 152-2. ×100.

[illegible] early divisions of
[illegible] lower right, partly
[illegible] Radial section of
[illegible] Transverse
[illegible] phelloderm
[illegible] Thick-walled
[illegible] preservation.

periderm. This fact should not, in our opinion, be construed as linking progymnosperms in any way with Filicales because Ophioglossales are not particularly closely related to Filicales (Bierhorst, 1971, p. 129). Bierhorst (1971, p. 151) suggests a derivation of Ophioglossales from certain progymnosperms or from coenopterids. Leclercq (1962, p. 54) wondered if progymnosperms might be an alliance parallel to gymnosperms and also to the Filicales. Our own studies have convinced us that progymnosperms are closely linked to the origin of the gymnosperms and not to the ferns. We prefer to think, in agreement with Bierhorst (1971, Fig. 28-10), that progymnosperms (Aneurophytopsida in his Fig. 28-10), are at the base of the gymnosperm complex and that Ophioglossales, Coenopteridales, and Filicales perhaps originated separately from a still earlier group, e.g., Rhyniopsida in Bierhorst's Fig. 28-10 or Trimerophytina, Banks (1968).

Periderm is characteristic of stems, branches and roots of gymnosperm, and is not normally produced by foliar organs (Esau, 1965, p. 339). Branches of Aneurophytales (sensu Scheckler and Banks, 1971b) have been considered homologous to megaphyllous leaves of pteridosperms (Andrews, 1961, p. 68; Bierhorst 1971, Chapt. 10). Other workers (e.g. Scheckler and Banks, 1971a), consider that only the ultimate appendages of the branches of *Tetraxylopteris* and *Triloboxylon* (Fig. 4) are homologous to leaves. We have found secondary xylem in all orders of branches but not in the ultimate appendages. We have similarly found periderm in all the older branches. It appears that periderm as presently known gives some support to the hypothesis that leaves in progymnosperms consist of the small, repeatedly bifurcated ultimate appendages. Extensive data from another order of progymnosperms, the Archaeopteridales, lead to the same conclusion (Carluccio et al., 1966; Banks, 1968; Beck, 1970, 1971). In this order the leaves have become planated and webbed and are therefore much more obviously leaves.

REFERENCES

Andrews, H. N., Jr. 1961. *Studies in Paleobotany*. New York. J. Wiley and Sons, Inc.

Arnold, C. A. 1930. The genus *Callixylon* from the Upper Devonian of Central and Western New York. *Pap. Mich. Acad. Sci.* **11:** 1-50.

Banks, H. P. 1968. The early history of land plants. In *Evolution and Environment: a symposium presented on the one hundredth anniversary of the foundation of Peabody Museum of Natural History at Yale University.* E. Drake (ed.). New Haven and London. 73-107.

Beck, C . B. 1957. *Tetraxylopteris schmidtii* gen. et. sp. nov., a probable pteridosperm precursor from the Devonian of New York. *Am. J. Bot.* **44:** 350-367.

——. 1960. The identity of *Archaeopteris* and *Callixylon*. *Brittonia* **12:** 351-367.

——. 1964. The woody, fern-like trees of the Devonian. *Mem. Torrey bot. Club.* **21:** 26-37.

——. 1966. On the Origin of Gymnosperms. *Taxon* **15:** 337-339.

——. 1970. The appearance of gymnospermous structure. *Biol. Rev.* **45:** 379-400.

——. 1971. On the anatomy and morphology of lateral branch systems of *Archaeopteris*. *Am. J. Bot.* **58:** 758-784.

Bierhorst, D. W. 1971. *Morphology of Vascular Plants*. Macmillan Co. New York.

Bonamo, P. M., and H. P. Banks. 1967. *Tetraxylopteris schmidtii*: its fertile parts and its relationships within the Aneurophytales. *Am. J. Bot.* **54:** 755-768.

Carluccio, L. M., F. M. Hueber, and H. P. Banks. 1966. *Archaeopteris macilenta*, anatomy and morphology of its frond. *Am. J. Bot.* **53:** 719-730.

Esau, K. 1965. *Plant Anatomy*. 2nd ed. J. Wiley and Sons, Inc. New York.

Fahn, A. 1967. *Plant Anatomy*. Pergamon Press, New York and Oxford.

Leclercq, S. 1962. Deux Plantes du Devonien. *Bull. Soc. Bot. Belg.* **95:** 51-59.

MATTEN, L. C., AND H. P. BANKS. 1966. *Triloboxylon ashlandicum* gen. and sp. n. from the Upper Devonian of New York. *Am. J. Bot.* 53: 1020-1028,

——, AND ——. 1967. Relationship between the Devonian progymnosperm genera *Sphenoxylon* and *Tetraxylopteris*. *Bull. Torrey. bot Club* 94: 321-333.

SCHECKLER, S. E., AND H. P. BANKS. 1971a. Anatomy and relationships of some Devonian progymnosperms from New York. *Am. J. Bot.* 58: 737-751.

——, AND ——. 1971b. *Proteokalon*, a new genus of progymnosperms from the Devonian of New York State and its bearing on phylogenetic trends in the group. *Am. J. Bot.* 58: 874-884.

Adv. Pl. Morph. 1972: 65–75

VASCULAR SUPPLY OF AXILLARY BUDS IN SOME DICOTYLEDONS

BY J. J. SHAH, J. D. PATEL AND I. L. KOTHARI

Department of Botany, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India

ABSTRACT

Vascular supply of axillary bud in 42 species of 22 dicotyledon families is investigated. Relationship of the bud traces with the various types of the node and the other vascular architecture present at the node is described. Unilacunar nodes show two bud traces but more than two bud traces can be present in tri- and multilacunar nodes. The five types of bud trace connections described by Ezelarab and Dormer (1963) are reviewed and three main types of bud trace connections in dicotyledons are described. Relationship with the bud trace connection and the branching behaviour of the strands flanking the leaf gap is described. The possible morphogenetic importance of this relationship is also discussed.

INTRODUCTION

Even though the vascular connections of the axillary buds with the axial primary vascular system of dicotyledons are a part of the nodal architecture, their studies have received hardly any attention in overall researches on stem primary vascular patterns in dicotyledons (Carlquist, 1962; Philipson and Balfour, 1963; Benzing, 1967a, b; Jensen, 1968). Histological types and significance of the bud trace connections in the phylogeny of the family Ranunculaceae and the Rhoeadales have been investigated (Ezelarab and Dormer, 1963, 1966). Recently some contributions on the vascular relationship of the bud traces with the axial vasculature at the node have been made in some selected dicotyledons (Shah and Unnikrishnan, 1968, 1969; Shah and Patel, 1969). We (Shah and Unnikrishnan, 1968, 1969; Shah and Patel, 1969) have previously shown that the types of bud trace connections described by Ezelarab and Dormer (1963) are not applicable in many cases. We wanted to investigate if there are any other basic types of bud trace connections in the nodal architecture and whether each of them is related with the type of the node. The present paper deals with 42 species belonging to 22 dicotyledon families.

MATERIALS AND METHODS

The data for the present investigation have been taken from the preparations of Drs K. Unnikrishnan, K. V. Poulose, K. S. Thulasy, P. H. V. Vasudeva Rao and J. D. Patel. Clearing of the material was carried out as described by Shah and Patel (1969) with some changes in concentration of NaOH solution. The schematic drawings are based on the cleared material as well as, in most of

---

Two of us (J.D.P. and I.L.K.) are thankful to the University Grants Commission for the award of the Senior and Junior Fellowship respectively.

the cases, on serial cross sections.

OBSERVATIONS

Nodal types.—Three major types of nodes, unilacunar, trilacunar and multilacunar, are present in the investigated species. However, the unilacunar nodes show one trace (Fig. 1A), two trace (Fig. 1N), three trace (Fig. 2B) and multitrace (Fig. 2F) conditions. Members of the same family and sometimes species of the same genus show varying nodal structures. Verbenaceae and Solanaceae show unilacunar nodes with one, two or multitrace conditions (Table 1). Different species of *Clerodendrum* show either two-trace or multitrace unilacunar node. Thirty three species have unilacunar node with varying number of leaf trace strands. Trilacunar node was found in six, and multilacunar in the remaining ones (Table 1 and Figs. 1-3).

Bud Traces.—Majority of the investigated dicotyledons show two bud traces. Nevertheless, there are three bud traces in *Cuminum cyminum* (Fig. 3D), four in *Muehlenbeckia platyclados* (Fig. 3G) and *Plantago ovata* (Fig. 3I), and more than four in *Foeniculum vulgare* (Fig. 3F).

Bud traces are associated with various vascular strands at the respective node. We have attempted to discuss the relationship of the bud traces with the various types of the node and the other vascular architecture at the node.

Unilacunar One Trace Node.—The species showing unilacunar one trace condition have two bud traces. In *Anisomeles ovata* (Fig. 1O) and *Duranta repens* (Fig. 1D) each of the bud traces is associated with the leaf trace sympodium (Esau, 1965a) which also gives rise to a leaf trace (mostly referred below as the trace of the subtending leaf) and one or two strands of the internode above. These internodal strands flank the gap and do not divide at the node. In *Cestrum diurnum* (Fig. 1I), *Ipomoea cairica* (Fig. 1A), *I. quamoclit* (Fig. 1B) and *Petrea volubilis* (Fig. 1H) the bud traces originate from the leaf traces. The strands flanking the leaf gap divide towards the gap in *Cestrum diurnum* (Fig. 1I) and *Petrea volubilis* (Fig. 1H) whereas, in *Ipomoea cairica* and *I. quamoclit* (Figs. 1 A, B) they divide away from the gap. The bud traces in *Carissa congesta* (Fig. 1J), *Cestrum nocturnum* (Fig. 1E), *Ehretia buxifolia* (Fig. 1K), *Maytenus emarginata*, *Memecylon umbellatum* (Fig. 1C), *Salvadora persica*, *Syzygium cumini* and *Vitex negundo* are associated with the strands flanking the leaf gap. In these species the flanking strands do not divide at the node. However, it must be mentioned that in *C. congesta*, *E. buxifolia*, *M. emarginata*, *M. umbellatum*, *S. persica* and *V. negundo* the axial vasculature does not resolve into a distinct eustelic pattern so that it is difficult to detect the division of a vascular strand (Table 1, shown with the mark '—').

Unilacunar Two Trace Node.—The bud traces are two. They originate either from the leaf traces or the strands flanking the leaf gap. In *Leucas aspera* (Fig. 1 Q) each of the bud traces is associated with the strand (trace sympodium) which gives rise to a leaf trace strand and a flanking strand of the gap. The latter unites with the strand flanking the opposite gap at the node and this union results into two strands, each flanking the gap. In *Capsicum annuum* (Fig. 1F), *Leonurus sibiricus* (Fig. 1S) the strands flanking the gap do not divide at the node but only give rise to bud traces. In *Ocimum americanum* (Fig. 1P) the strands flanking the gap divide with their branches oriented away from the gap. However,

TABLE I

BUD TRACE RELATIONSHIP AT THE NODE IN THE INVESTIGATED DICOTYLEDON PLANTS

Species (I)	Family (II)	Nodal type (III)	Number of bud traces (IV)	Type of bud trace connection (V)	Behaviour of the strands flanking the leaf gap (VI)
Amaranthus tricolor L.	Amaranthaceae	UThT	2	I	DAG
Anisomeles ovata R. Br.	Labiatae	UOT	2	III	DN
Bougainvillea spectabilis Willd.	Nyctaginaceae	UMT	2	I	DN
Capsicum annuum. L.	Solanaceae	UTT	2	I	DN
Carissa congesta Wight.	Apocynaceae	UOT	2	I	—
Cestrum diurnum L.	Solanaceae	UOT	2	II	DTG
C. nocturnum L.	,,	UOT	2	I	DN
Clerodendrum inerme Gaertn.	Verbenaceae	UTT	2	II	DTG
C. phlomidis L.	,,	UTT	2	II	DTG
C. splendens G. Don.	,,	UMT	2	I	DN
Clerodendrum sp.	,,	UTT	2	II	DTG
Cuminum cyminum L.**	Umbelliferae	TL	3	II	DTG
Datura innoxia Mill.	Solanaceae	UThT	2	I	DN
Duranta repens L.	Verbenaceae	UOT	2	III	DN
Ehretia buxifolia Roxb.	Boraginaceae	UOT	2	I	—
Euphorbia tirucalli L.	Euphorbiaceae	TL	2	I	DN
Feronia limonia L.	Rutaceae	TL	2	I	—
Foeniculum vulgare Mill.	Umbelliferae	ML	4↑	II	—
Hibiscus cannabinus L.	Malvaceae	TL	2	I	—
Ipomoea carica Sw.	Convolvulaceae	UOT	2	II	DAG
I. quamoclit L.	,,	UOT	2	II	DAG
Leonurus sibiricus L.	Labiatae	UTT	2	I	—
Leucas aspera (Willd.) Spreng.	,,	UTT	2	III	DTG
Lycopersicon lycopersicum (L) Santapau	Solanaceae	UMT	2	I	—
Maytenus emarginata (Willd.) Ding Hou.	Celastraceae	UOT	2	I	—
Memecylon umbellatum Burm.	Melastomaceae	UOT	2	I	—
Mentha spicata L.**	Labiatae	UTT	2	II	DTG
Michelia champaca L.	Magnoliaceae	ML	2	I	DN
Muehlenbeckia platyclados Meissn.	Polygonaceae	ML	4	III	—
Ocimum americanum L.	Labiatae	UTT	2	I	DAG
Petrea volubilis L.	Verbenaceae	UOT	2	II	DTG
Plantago ovata Forsk.	Plantaginaceae	TL	4	II	DTG
Salvadora persica L.	Salvadoraceae	UOT	2	I	—
S. oleoides Decne.	,,	UMT	2	I	—
Santalum album L.	Santalaceae	UThT	2	I	DN
Solanum melongena L.	Solanaceae	UThT	2	I	DN
Syzygium cumini L.	Myrtaceae	UOT	2	I	DN
Tectona grandis L.	Verbenaceae	UMT	2	III	—
Thunbergia erecta T. Anders.	Acanthaceae	UThT	2	I	DN
Trigonella foenum-graecum L.	Papilionaceae	TL	2	I	DN
Vitex negundo L.	Verbenaceae	UOT	2	I	—
Withania somnifera (L.) Dunal.	Solanaceae	UThT	2	II	DTG

**species shows variations in nodal vasculature, and only one type has been given here.

DAG, divide away from the leaf gap; DN, do not divide; DTG, divide towards the leaf gap; ML, multilacunar; TL, trilacunar node; UMT, Unilacunar multitrace node; UOT, Unilacunar one trace node; UTT, unilacunar two trace node; UThT, unilacunar three trace node; I, II, III, types of bud trace connection (see in discussion).

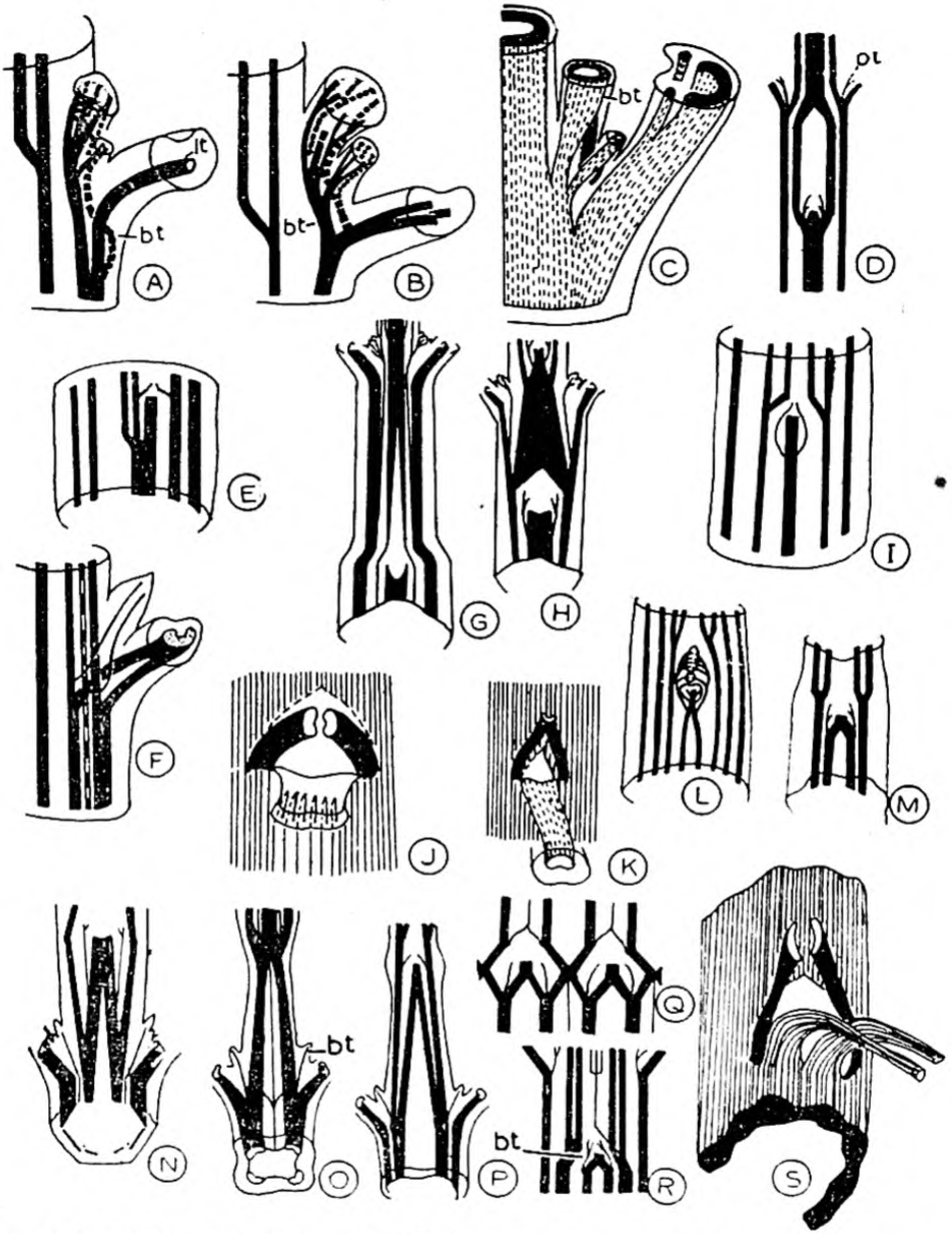


FIG. 1 A—S. Schematic representation of bud trace connections at the node. A. *Ipomoea cairica.* B. *I. quamoclit.* C. *Memecylon umbellatum.* D. *Duranta repens.* E. *Cestrum nocturnum.* F. *Capsicum annuum.* G. *Syzygium cumini.* H. *Petrea volubilis.* I. *Cestrum diurnum.* J. *Carissa congesta.* K. *Ehertia buxifolia.* L. *Clerodendrum inerme.* M. *C. phlomidis.* N. *Clerodendrum* sp. O. *Anisomeles ovata.* P. *Ocimum americanum.* Q. *Leucas aspera* R. *Mentha spicata.* S. *Leonurus sibiricus.*

bt, bud trace; lt, leaf trace.

the strands flanking the gap divide towards it in *Clerodendrum inerme* (Fig. 1L). *C. phlomidis* (Fig. 1M) and *Mentha spicata* (Fig. 1R) where the bud traces arise from the trace of the subtending leaf.

Unilacunar Three Trace Node.—The two bud traces arise from the strands flanking the leaf gap in *Amaranthus tricolor* (Fig. 2E), *Datura inoxia, Santalum album* (Fig. 2B), *Solanum melongena* (Fig. 2D) and *Thunbergia erecta* (Fig. 2C). In the last four species the strands flanking the gap do not divide at the node whereas in *A. tricolor* they divide away from the gap at the node. In *Withania somnifera* (Fig. 2A) the two bud traces are derived from the lateral leaf trace strands whereas, the strands flanking the gap divide towards the gap.

Unilacunar Multi Trace Node.—In *Bougainvillea spectabilis* (Fig. 2G), *Clerodendrum splendens* (Fig. 2I), *Lycopersicon lycoperiscum* (Fig. 2H) and *Salvadora oleoides* the strands flanking the leaf gap do not divide and give rise to bud traces. In *Tectona grandis* (Fig. 2F) a girdle strand, formed between the two strands on either flank of the leaf gap, gives rise to two bud traces.

Trilacunar Node.—*Euphorbia tirucalli* (Fig. 3C), *Feronia limonia* (Fig. 3B), *Hibiscus cannabinus* (Fig. 3A) and *Trigonella foenum-graecum* (Fig. 3E) show two bud traces whereas, *Cuminum cyminum* (Fig. 3D) shows three and *Plantago ovata* (Fig. 3I) shows four bud traces. In the group of first four species, the bud traces arise from the strands flanking the median gap, and these strands do not divide at the node. In *Cuminum cyminum* all the three leaf traces give off one bud trace each where the strands flanking the leaf gaps divide. In *Plantago ovata* (Fig. 3I) each of the bud traces is derived from the lateral leaf traces whereas, two more bud traces are derived from the median leaf trace. The strands flanking the gaps in this case also divide towards the gaps.

Multilacunar node.—There are two bud traces in *Michelia champaca* (Fig. 3H), four in *Muehlenbeckia platyclados* (Fig. 3G) and more than four in *Foeniculum vulgare* (Fig. 3F). The two bud traces in *M. champaca* are derived from the strands flanking the median leaf gap where the flanking strands do not divide at the node. All the leaf trace strands in *F. vulgare* give off two bud traces each. The flanking strands do not divide. A plexus formed by the union of a number of strands of axial vasculature at the node is a common source of the leaf and bud traces in *M. platyclados* (Fig. 3G).

Discussion

The histological identity of the bud traces has not been uniformly recognised. The bud traces which are two in initial developmental stages become an arc, a horseshoe-shaped structure and a vascular ring in *Syringa* (Garrison, 1949). Clowes (1961) also believes that in *Euptelia polyandra* the bud traces unite to form a half cylinder of procambium. If the branch supply consists of two traces, they unite within a short distance, forming a complete ring (Eames and MacDaniels, 1947). However, Esau (1965a) defines the bud trace as "a vascular bundle in the main stem extending between its connection with the vascular tissues of the branch and that with another vascular unit in the main stem." Shah (1968) considers that "ontogenetically the term 'bud trace' should be used for two or more vascular strands which form a link between the vascular system of the axis and the primary vascular system of the bud", and that "the structures, described

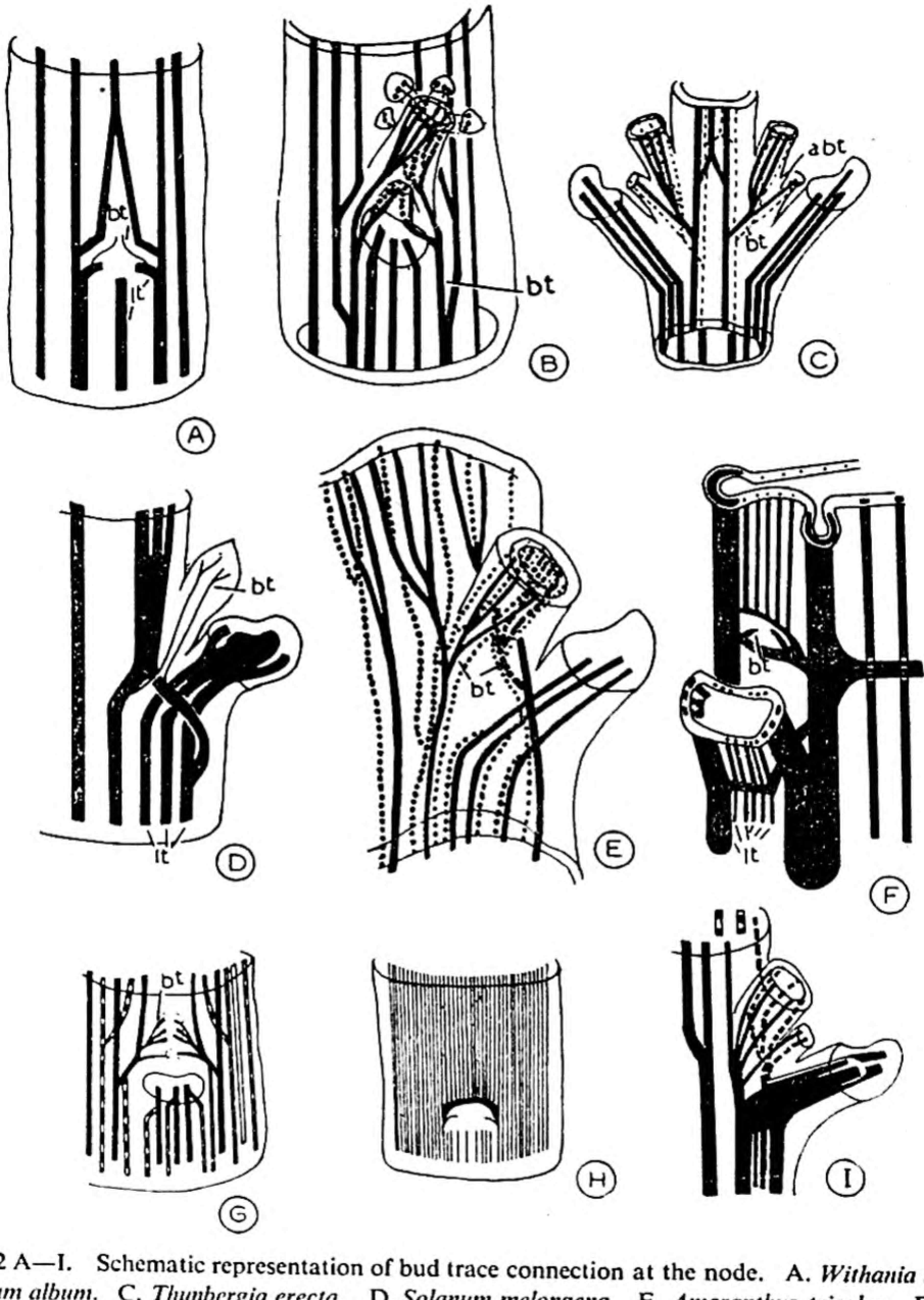


FIG. 2 A—I. Schematic representation of bud trace connection at the node. A. *Withania somnifera*. B. *Santalum album*. C. *Thunbergia erecta*. D. *Solanum melongena*. E. *Amaranthus tricolor*. F. *Tectona grandis*. G. *Bougainvillea spectabilis*. H. *Lycopersicon lycopersicum*. I. *Clerodendrum splendens*.

abt, accessory bud trace; bt, bud trace; lt, leaf trace.

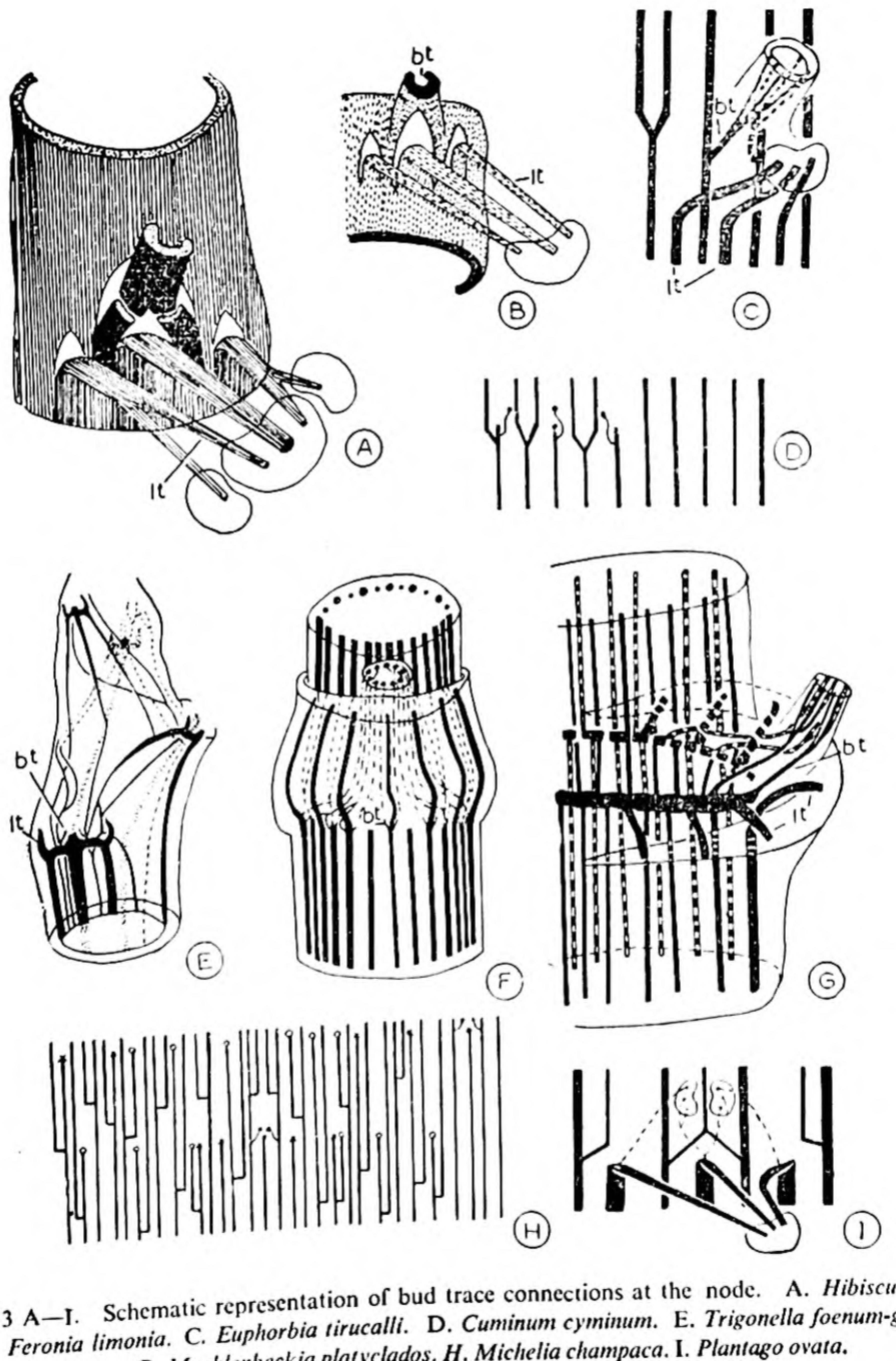


FIG. 3 A—I. Schematic representation of bud trace connections at the node. A. *Hibiscus cannabinus*. B. *Feronia limonia*. C. *Euphorbia tirucalli*. D. *Cuminum cyminum*. E. *Trigonella foenum-graecum*. F. *Foeniculum vulgare*. G. *Muehlenbeckia platyclados*. H. *Michelia champaca*. I. *Plantago ovata*.

bt, bud trace; **lt**, leaf trace.

as arcs or horseshoe-shaped or ring-like are the intermediate histogenic expressions of the primary vascular system of the bud". We have in the present investigation adopted the concepts of Esau (1965a) and Shah (1968) for the identification of the bud traces. In our observations we have described the bud traces as associated with, derived from ……etc., but this description does not indicate in any way the ontogenetic history of the bud traces in the investigated species.

The present investigation confirms that generally two bud traces are common in dicotyledons, but more than two bud traces are also reported as also observed in this investigation (Dormer, 1950; Ezelarab and Dormer, 1963, 1966; Esau, 1965a; Devadas and Beck, 1971). In no case unilacunar nodes show more than two bud traces in the investigated species but more than two bud traces can be present in trilacunar and multilacunar nodes. Ezelarab and Dormer (1963) have reported many branch traces related to a single gap (Fig. 4 E).

The present investigation shows that the bud traces, usually, arise either from the subtending leaf trace or the strands flanking the gap of the subtending leaf (Ezelarab and Dormer, 1963). However, according to Esau (1965a) branch traces, like leaf traces can be traced in the lower internodes and they form a component of the axial vasculature. In *Azima tetracantha* (Kshetrapal and Tiagi, 1967) this condition is reported. Similar condition is also observed in the illustration of the vascular pattern of *Cassia didymobotrya* (Devadas and Beck, 1971). In the species investigated the bud traces arise at their respective nodes but Devadas and Beck (1971) reported that they extend from one to five or more internodes in species of *Cassia* and *Potentilla*.

Very little attention has been given to the relationship of bud traces with the axial vasculature. Ezelarab and Dormer (1963) have described five types of bud trace connections with the axial vasculature in the family Ranunculaceae. (Fig. 4A-E). Their Normal and *Petasites* types refer to in the tri- and multilacunar node respectively. In the former the branch traces arise from the strands bordering the median leaf gap and in the latter they arise from the bundles bordering the median and lateral leaf gaps (Fig. 4A and B respectively). Their *Berberis* and *Lepidium* types respectively refer to unilacunar single (Fig. 4C) and multi-trace (Fig. 4E) condition, and *Thalictrum* type refers to multilacunar node (Fig. 4 D). In all these three types branch traces arise either only from the median leaf trace (*Berberis* type) or from the median and lateral leaf traces.

We believe that any classification of bud trace connections with axial vasculature related with the nodal architecture will be untenable (Shah and Unnikrishnan, 1968). For example, *Petasites* type is reported in a multilacunar node, and mostly with sheathing leaf bases (Dormer, 1950). *Foeniculum*, as reported above, shows multilacunar node with sheathing leaf base but its bud trace connection resembles *Thalictrum* type of Ezelarab and Dormer (1963). On the other hand *Muehlenbeckia* shows a vascular girdle at the node as reported in *Petasites* (Dormer, 1950) but its formation is quite different and its bud trace connection does not resemble any of the types of Ezelarab and Dormer (1963). Elaborating the ideas of Shah and Unnikrishnan (1968) which were with reference to a few members of the families Verbenaceae and Labiatae, we now propose that bud trace connection is of following main types in dicotyledons:

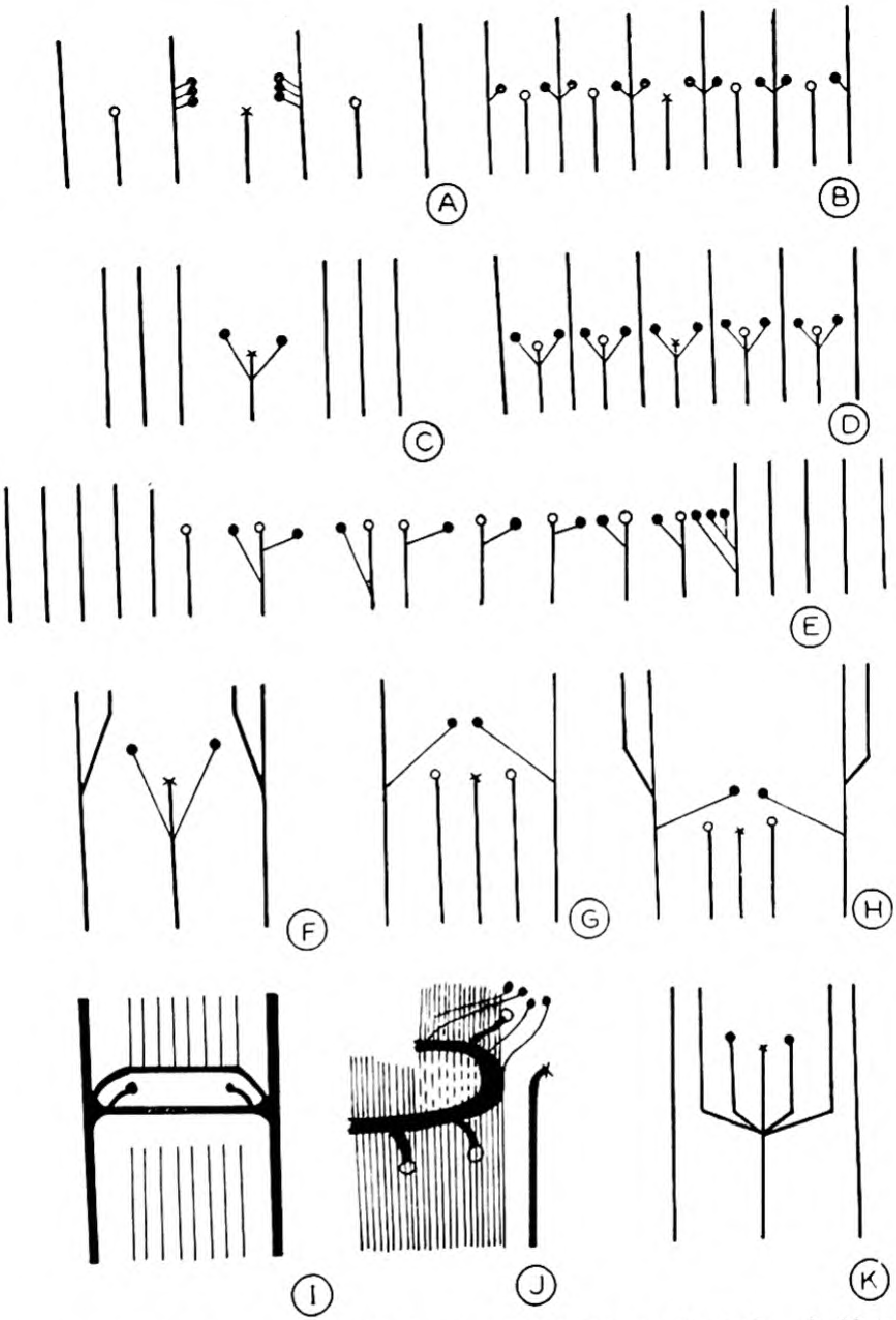


FIG. 4 A—K. Different types of bud trace connections at the node. A. Normal-condition. B. *Petasites*-condition. C. *Berberis*-condition. D. *Thalictrum*-condition. E. *Lepidium*-condition. F. Bud traces associated with the leaf trace, where the strands flanking the leaf gap divide towards the gap. G. Bud traces associated with the strands flanking the leaf gap which do not divide at the node. H. Flanking strands divide away from the gap and give rise to the bud traces at the node. I. Bud traces arising from the girdle. J. Bud traces and leaf traces arising from nodal plexus at the node. K. A common strand gives rise to the leaf trace, bud traces and the strands of the internode above. (A-E. Redrawn from Ezelarab and Dormer, 1963.)

Strands with *'X'* and *empty dots* represent the median and lateral leaf traces respectively, and those with *dark dots* represent the bud traces.

TYPE I.—Bud traces are associated with the vascular strands that are not the traces of the subtending leaf.

TYPE II.—Bud traces are associated with the traces of the subtending leaf.

TYPE III.—Bud traces are associated with the strand (leaf sympodium of Esau, 1965a) which gives rise to the axillant leaf trace and the strand/s traversing the internode above.

Previously we (Shah and Patel, 1969) tried to show some relationship with the origin of the bud trace connection and the branching behaviour of the strands flanking the axillant leaf gap in some Solanaceae species. Considering many dicotyledons belonging to diverse families, we come to the following conclusions:

(*a*) The bud traces arise above, below or at the same level with that of the trace of the subtending leaf when it enters the petiole (Figs. 1-3).

(*b*) The strands flanking the leaf gap do not divide if they are connected with the bud traces (Fig. 4G).

(*c*) If the strands mentioned in (*b*) divide, their branching is away from the axillant leaf gap (Fig. 4H).

(*d*) If the bud traces are connected with the traces of the subtending leaf, the strands flanking the axillant leaf gap have branches towards this leaf gap (Fig. 4F). However, *Ipomoea cairica* and *I. quamoclit* do not show this condition.

(*e*) Sometimes other patterns are also observed, which cannot be resolved into-*b-d*, but they certainly can be classified into the three categories of bud trace connections described above (Table I).

In *Tectona* the bud traces arise from a horizontal strand which arises from a sympodial strand that also flanks the leaf gap (Fig. 4I). In *Muehlenbeckia,* the bud traces, leaf traces and the strands of the internode above arise from the nodal plexus (Fig. 4G). In some species the bud traces arise from a strand connected with the trace of the subtending leaf and the strand of the internode above (Fig. 4K).

The most intriguing question is about the morphogenetic factor/s that determine the types of the bud trace connections with the axial vasculature. In a number of dicotyledons, the origin and development of the axillary bud trace procambium is reported acropetal, though basipetal and bidirectional procambium development is also reported (Esau, 1965b; Cutter, 1971).

What makes the bud trace procambium to develop as a leaf trace? Here the bud trace like the leaf trace is an extension of the axial strand at the node. The formation of the leaf and its trace procambium at the shoot apex is regarded as complimentary aspects of a single process (Allsopp, 1964). The extension of the bud trace procambium may be a result of the morphogenetic interaction between the bud, shoot apex and the axillant leaf. They may be regarded as "physiological sinks" which initiate the procambial pathway of translocation of metabolites (Wardlaw, 1968).

In the investigated species the bud trace develops as a branch of the trace of the subtending leaf or of a strand flanking the leaf gap (referred above as three types of bud trace connections). The morphogenetic situation is different from that of the above. From a particular locus of the existing strand in the stem, the bud trace procambium develops as a branch, if its development is acropetal, as in many of the investigated species. In this situation we may conceive of the existence of both acropetal and basipetal metabolic gradients between the bud apex and the subjacent leaf and axial regions (Wardlaw, 1968; Cutter, 1971). A particular gradient determines the locus on the existing strand from where the acropetal differentiation of the bud trace procambium will begin. Our observations on the relationship between the origin of bud trace connection and the branching behaviour of the strands flanking the leaf gap may have some bearing in establishing a particular gradient.

REFERENCES

Allsopp A. 1964. Shoot morphogenesis. *Ann. Rev. of Pl. Physiology* **15**: 225-254.

Benzing D. H. 1967a. Developmental patterns in stem primary xylem of woody Ranales. I. Species with unilacunar nodes. *Am. J. Bot.* **54**: 805-813.

——. 1967b. Developmental patterns in stem primary xylem of woody Ranales. II. Species with trilacunar and multilacunar nodes. *Ibid.* **54**: 813-820.

Carlquist, S. 1962. *Comparative Plant Anatomy*. Holt, Rinehart and Winston New York.

Clowes. F. A. L. 1961. *Apical Meristems*. Blackwell Scientific Publications, Oxford.

Cutter, E. G. 1971. *Plant Anatomy: Experiment and interpretation. Part 2. Organs*. Edward Arnold, London.

Devadas, C., and C. B. Beck. 1971. Development and morphology of stelar components in the stems of some members of the Leguminosae and Rosaceae. *Am. J. Bot.* **58**: 432-446.

Dormer, K. J. 1950. Observations on the vascular supply to axillary branches. *New Phytol.* **49**: 36-39.

Eames, A. J., and L. H. MacDaniels. 1947. *An Introduction to Plant Anatomy*. McGraw-Hill, New York.

Esau. K. 1965a. *Plant Anatomy*. John Willey and Sons, New York.

——. 1965b. *Vascular Differentiation in Plants*. Holt. Rinehart and Winston, New York.

Ezelarab, G. E., and K. J. Dormer. 1963. The organization of the primary vascular system in Ranunculaceae. *Ann. Bot. N.S.* **27**: 23-38.

——, and ——, 1966. The organization of the primary vascular system in the Rhoeadales. *Ann. Bot.* **30**: 123-132.

Garrison, R. 1949. Origin and development of axillary buds: *Syringa vulgaris* L. *Am. J. Bot.* **36**: 205-213.

Jenson, L. C. W. 1968. Primary stem vascular patterns in three subfamilies of the Crassulaceae. *Am. J. Bot.* **55**: 553-563.

Kshetrapal. S., and Y. D. Tiagi. 1967. A contribution to the nodal anatomy of *Azima tetracantha* Lamk. *Bot. Bull. Acad. Sinica* **8**: 171-183.

Philipson, W. R., and E. E. Balfour. 1963. Vacscular patterns in Dicotyledons. *Bot. Rev.* **29**: 382-404.

Shah, J. J. 1968. Axillary bud traces in certain dicotyledons. *Can. J. Bot.* **46**: 169-175.

——, and J. D. Patel. 1969. Vascular interrealtionships in some Solanaceae members. *Biologia Pl.* **11**: 329-333.

——, and K. Unnikrishnan. 1968. Vascular connections of axillary buds in some members of the Verbenaceae and the Labiatae. *Proc. Indian Acad. Sci.* **68B**: 284-288.

——, and ——. 1969. Bud trace connections in *Tectona grandis*. L. *Curr. Sci* **38**: 298-299.

Wardlaw, C. W. 1968. *Morphogenesis in Plants*. Methuen and Co., London.

Adv. Pl. Morph. **1972: 76–84**

DESCRIPTIVE WRITING WITH SPECIAL REFERENCE TO PLANT ANATOMY

BY C. R. METCALFE

Formerly Keeper of the Jodrell Laboratory, Royal Botanic Gardens, Kew, U. K.

ABSTRACT

Well prepared, concisely worded descriptions are a more important part of biological communication than is sometimes realized today. In the face of proliferating publications, intending authors must make sure, before they begin to write, for whom their message will be important and whether the cost of committing it to print is justified. Restricted circulation of specialized data is sometimes achieved more effectively and economically in mimeographed brochures than in articles printed in journals.

INTRODUCTION

Professor V. Puri to whom this volume is dedicated, has been well known for many years for his morphological writing, sespecially for those dealing with floral morphology and the evolution of angiosperms. His publications reveal his broad interest in these subjects and he has a marked capacity for expressing himself in simple language. He is a strong advocate of morphological teaching, especially as a groundwork for more specialized studies. He has also pleaded that this teaching should be presented only by those who are really well informed and who realize that elementary instruction calls for careful preparation and high qualities of exposition.

My own work on comparative anatomy, like Professor Puri's comparative morphological studies, has demanded a great deal of descriptive writing. It seems appropriate, therefore, in a volume dedicated to Professor Puri, to discuss some of the problems that face authors of descriptive articles.

Throughout my own work it has often been difficult to decide how best to present a mass of histological facts in a form in which they can be easily assimilated by the reader. There has in the first place been a continuous struggle between the alternatives of describing a few species in great detail as opposed to giving a more superficial treatment to a much wider spectrum of the flowering plants. These are problems that have to be faced by all morphologists, not least by those whose interest lies in histology. Whilst recognizing the great value of intensive studies on a few or even on a single species my own personal inclination has, for the most part, been towards broad surveys of numerous taxa, for it is my belief that without investigations that are wide in scope we may draw wrong conclusions. This is because, after completing a detailed survey of a restricted number of species, there is always a danger that we may jump to the conclusion that our findings will apply equally to other groups of organisms that we have not actually examined, only to find that this is not necessarily so when taxonomically wider investigations are subsequently undertaken. My studies in comparative anatomy have made me realize that histological criteria that are of great taxonomic or descriptive value in members of one particular taxonomic group such as a family, are not always of equivalent significance in others.

THE EXPLOSIVE PROLIFERATION OF PUBLICATIONS

The Washington D.C. (U.S.A.) correspondent of 'Nature' (2) recently wrote as follows:—'The average life scientist (biologist) produces not one book every two decades but more than four publications a year, two of them being full length research articles'. This remark was inspired by a report of a committee set up by the National Academy of Sciences under their President Dr. Philip Handler. The committee predicted that the publication of journals will be made obsolete within a short period of years by the computer console. Methods of communicating by computer are, however, likely to be developed independently in different parts of the world. In the early stages there will be consequential problems in translating the findings of one locally developed computer technique into another developed elsewhere. The Handler committee therefore allows that an additional period of ten years will be needed to provide communication links between the computer systems that may be in vogue in, for example, different continents. In this same note in 'Nature' it is stated that although 13,000 biological journals exist, the academy committee claims that more than 90 per cent of the most significant published articles are confined to about 1000 journals. Furthermore it is rather alarming to read that although 'Biological Abstracts' covered some 7,400 journals in 1968 'most of these are unlikely to publish anything that will materially advance the progress of science'.

The Handler committee are not alone in predicting that the computer console will take over from the published journal some at least of the information storage and retrieval for which we at present rely on printed matter. This prediction raises the immediate problem of how we are to act during the next few decades during which computer techniques capable of dealing with descriptive matter are expected to be developed. Do we cease to publish and await the computer age, retaining our results in the form of notes which can be computerized when our successors have access to the necessary machines. It seems to me that, even if we were to behave in such a stupid way, there would be no guarantee that our successors will take the trouble to make our current findings widely known. Surely they will first place their own results on record before taking much notice of ours and so the research for the next few decades might be lost for ever. But then we might ask ourselves whether this would matter very much if we accept the view that the bulk of current publications in biology are not going to add materially to the advance of science!

It is more than likely that, in the computer age, the translation of descriptive data into a form in which they can be handled on a machine will be entrusted to specialized technicians. Conversely, data given to us by the machine when we ask it questions will also need to be translated into the everyday language of descriptive biology. Since we can scarcely hope that there will be as many computer technicians as there are specialized branches of knowledge, it seems that a failure of accurate communication or translation will always be possible. Until the method has been used extensively we shall not know, but one would think that mistakes could be numerous and made very rapidly because the computers work so quickly. Indeed the speed with which we will be able to obtain results will in itself be a disadvantage. When mistakes are made at the frontiers of knowledge they may remain undetected unless adequate methods for checking the veracity of computer statements are always applied.

THE BACKGROUND AND PURPOSES OF DESCRIPTIVE WRITING

Because morphological and histological research is bound to continue whilst the unknown quantities about computer techniques are being worked out, we shall have still, for some time yet, to store, retrieve and discuss information by methods that are in current use to-day.

A large part of scientific investigation is very rightly experimental, but many biologists add to knowledge by observational exercises. Indeed, we may note that a large proportion of the data recorded by some of the greatest figures in biology such as Linnaeus, Darwin and Wallace, to mention only a few, were more frequently obtained by making observations than by conducting experiments. The distinction between results obtained by experimenting and observation respectively is, however, less clear cut than is sometimes supposed. This is because observational studies record the results of nature's own experiments. Natural experiments are often more difficult to interpret than those designed by man, because of the number of interdependent influences that are simultaneously operating on the organisms under observation. The correct interpretation, including written descriptions of what we observe, therefore, demands a specially high order of ability on the part of the investigator.

Observations may refer to the external form or internal structure of organisms. Other observational results may be concerned with such subjects as ontogenetic development, or with the mode of life of organisms in their natural environment. No matter in which particular field our observational interests may lie, or at what magnifications we usually work, the first essential is to record our results concisely, unambiguously and in a form that makes for speedy reference. Although we may be entering the computer age, books and journals are likely to remain the most important sources of reference material in the years that lie immediately ahead simply because they are convenient and easy to use. Moreover, they can be consulted at any time, unlike the computer technician, who will almost certainly be engaged on somebody else's work when his services are most urgently needed for our own.

In spite of the multifarious publications that exist, the average research worker does not often need to have access to more than a relatively small number of publications at any one moment. Consequently, existing methods of library working are not so hopelessly inadequate as we are sometimes led to believe, especially in these days when there are improved facilities for library interchange and for photocopying. Even when we are annoyed by unavoidable delays in securing the literature that we require this allows us the benefit of a little more time for thought.

SELECTION AND STORAGE OF MATERIAL TO BE DESCRIBED

It is perhaps hardly necessary to emphasize that specimens and microscope slides on which descriptive writings are based must remain available for future reference. Unless subsequent investigators are able to see material that has been previously studied, there can be no means of verifying facts and conclusions when this becomes necessary, or even of confirming the identity of the previously investigated material. So often, when writing a descriptive article, the author feels that his material is so familiar and well known that no confusion about its identity can possibly arise in the future. However, we should remember that a survey of the literature written during the past two decades shows that taxonomic revisions and nomenclatural name changes are still very numerous. This means that, besides the question of whether the material was correctly named at the time of a previous investigation, there is also a chance that the names may have since been changed for taxonomic or nomenclatural reasons. A state of taxonomic and nomenclatural flux will inevitably persist for many years to come, and indeed it would be wrong to hinder taxonomic evolution by bringing systematic studies to a halt.

The traditional method of preserving material for future reference where histological investigations are concerned is in the form of herbarium specimens to serve as vouchers. The existence of voucher material will serve no useful purpose, however, unless the authors of published articles arrange for their material to be deposited at a recognized research centre and indicate clearly in their publications where their voucher material is located.

Voucher material in the form of herbarium specimens is not of much value if, for example, one is describing histological details that become obscure when tne specimens are dried, usually because the cells become disorganized or distorted. With material that cannot be dried without distortion it is necessary for specimens to be preserved in a liquid preserving and fixing fluid such as formalin acetic alcohol. Then again there is the problem of plants with organs that are too large to be preserved by orthodox herbarium methods. To take a familiar example, a single frond of a coconut palm may be so long, heavy and generally unmanageable that it cannot easily be carried intact by a single person. How, one might ask oneself, is reference material of such a large specimen to be preserved. Here the only feasible method is to pickle selected small portions in a liquid preservative. All pickled material, like the herbarium vouchers, will need to be deposited at a recognized centre where facilities must be made available for it to be stored and cared for.

Another well known example of material that presents storage problems is afforded by timber specimens. Descriptions of wood are often based on specimens of which there are no means of checking the identity, because foliage, flowers and fruits from the same tree are no longer available. In facing this problem it is often suggested, with good reason, that herbarium material and wood specimens from the same tree should both be preserved together for record purposes. To do this entails much labour and care, but in spite of this difficulty, both types of material should be collected whenever circumstances permit. In the writer's experience, however, misidentifications can sometimes still occur even when herbarium specimens and wood samples from the same source are available. This is sometimes due to confusion over the reference numbers on the wood specimens and the herbarium vouchers respectively. Another cause for misidentification that could also arise is because a herbarium botanist might make a mistake in identifying the material.

The risk of a herbarium botanist doing this may well be rather small, but we must remember that even herbarium botanists are human, and therefore liable to error. There is a widespread feeling that when once a specimen has been introduced into a herbarium and given a name and reference number this action must somehow confer on that specimen absolute reliability as a standard for comparison. Whilst we would all agree that the chances of a specimen of this kind being authentically named are very high, a moment's reflection will indicate that this may not always be so. Mistakes in indentification can sometimes be brought to light by making comparisons betwen wood specimens purely at the histological level. The canons of timber identification at the histological level have now been so firmly established by prolonged and repeated comparisons that a well qualified wood anatomist can sometimes tell a herbarium botanist if his identifications are wrong or suspect. This all goes to show, how desirable it is to recognize that taxonomy should be based on a much wider range of characters than was at one time deemed to be necessary. It is not a question of one discipline having a greater validity than another in solving taxonomic problems; it is simply that a valid conclusion is more likely to be reached when several disciplines, rather than a single discipline, are applied to solving a specific problem. The value of an interdisciplinary approach to taxonomic problems is already much more widely recognized than it used to be. There is room for further improvement, however, and a general recognition of the value of co-operation between specialists would be still more useful.

THE PRACTICE OF WRITING

A research worker confronted with the task of preparing his first paper generally hesitates to make a start for as long as he can avoid doing so. The reason for this is that very few of us have such good opportunities to practise writing to-day as were available to previous generations of biologists. In our everyday lives we use the telephone to avoid writing letters. Wherever we go we are expected to fill in forms rather than to express ourselves in written sentences. School classes are now so large that teachers no longer have the time to give adequate instruction in composition. Is it surprising in these circumstances that students can sometimes proceed to a university with but limited experience in writing prose? When at the university students are so hard pressed by the need to absorb information, chiefly in the form of factual data, that it is only a small minority who make any serious attempt to discover the best way of expressing their ideas by the written word.

Nevertheless the day arrives when the need to write a thesis or an article must be faced. A student in this position generally turns to contemporary publications to see how others are expressing their ideas. Unfortunately, the student will find that many of the articles are written in the form of jargon which is accepted in scientific circles as the normal medium for communication. When trying to fall into line with the current practice, a student will perpetuate the circumlocutions, that take the place of simple sentences. He will use needlessly long words, replace adjectives by abstract nouns, and he will adopt the passive rather than the active voice. The result of this strict adherence to scientific convention is that the whole subject becomes much more complicated than it need be, and the exchanges of factual information or thought between author and reader are impaired.

There is no royal road to writing effectively because the art of self expression varies very much from one individual to another. Of one thing we may be certain, however, namely that to communicate facts or ideas in writing in the simplest possible way calls for much thought and practice. Also we should not feel constrained to adhere rigidly to a mode of writing that is currently orthodox if we can devise improvements. A biology student should face up to this long before the time comes for him to write a thesis or an article intended for publication. He should realize, from the time when his studies begin, that he will sooner or later, have to communicate with his fellow research workers through the medium of the printed word. If he can do this in simple language he will earn the lasting gratitude of his readers and at the same time increase his chances of improving his scientific status simply because his writing will be easy to understand. One way to achieve this aim is for the student to devote a little time, it need not be more than a few minutes, if not every day then at least at frequent intervals, to writing simple paragraphs that might form part of a published article. There is no need to keep these jottings; it is the act of producing them that is important because it provides practice in handling words and in constructing sentences and paragraphs.

An intending research worker who has taken the trouble to acquire skill in writing will find the task of preparing his early publication much easier. He will also soon be on good terms with editors of journals because his articles will be concise and to the point and the expense of needless printing will be avoided.

We are, I take it, all aware that it is preferable to say 'No' rather than 'The answer to the question is in the negative'. This is a very elementary example of needless expansion. We must now pass on to consider other examples of passages that have been made needlessly complicated by their authors. The following quoted sentences show that even those who makes regulations concerning examinations do not always express themselves in the simplest possible terms. 'In the case of every candidate who is placed in the first class the class-list will show by some convenient mark (i) the section or sections for proficiency in which he is placed in the first class and (ii) the section or sections (if any) in which he has passed with special distinction'. Would not the following much shorter version be an improvement ? 'Convenient marks on the class-list can be used to show the section or sections in which each first class student is proficient and whether he has gained special distinction in any of them.'

Some years ago a well known botanist, now deceased, wrote a lengthy article which included numerous complex passages of which the following may be taken as an example. "Problems of growth forced reproduction towards the end of the season, whether sexual or asexual reproduction, and a delayed dispersal of seeds made the service of the wind profitable before the family spread from a subtropical life to tropical forests. All along the history of progressive size there had been the possibility of slipping out of the struggle against the young trees by accepting the shade of rocks instead, though this involved accepting inferior conditions at the soil". Passages such as this make descriptive botany dull and depressing, and nobody could blame a beginner if he were to be discouraged by this style or writing. I will leave it to readers of this article to decide for themselves just what the author of this quoted passage intended to say.

For most purposes simple prose, suitably supported by illustrations and graphs, is the obvious written medium for describing structure. Taxonomists, faced with the problem of describing vast numbers of organisms, have, however, for many generations, adopted abbreviated forms of descriptive writing which amount almost to botanical shorthand. Normal sentences are avoided, particularly by the elimination of verbs. The purposes for which this mode of writing can be adopted is limited by its lack of flexibility, but it is highly effective when used in the right place and for purely descriptive purposes. It can be used just as well to describe the histology as the external form of a plant and it saves an immense amount of space. To write botanical shorthand requires just as much or even more practice than normal prose, simply because the beginner is less familiar with it. It is, however, well worth while for descriptive writers to learn how to express themselves in this medium because it is so useful where numerous taxa are involved. With care, nothing of importance need be omitted.

Ph. D. theses are often much too verbose. We were recently reminded of this by Halstead (1) in a letter to 'Nature' which includes the following words:—"An essential part of training in research should be preparation for publication, yet the Ph. D. candidate is allowed to indulge in a verbose expansiveness that no editor of a scientific journal would contemplate for a moment". Halstead concludes "On the grounds of cost-effectiveness, obligation to the scientific community and self-interest of the candidate, the present system of preparing Ph. D. theses should be discarded at the earliest opportunity."

To give an example based on my own experience, I was recently asked to examine a Ph. D. candidate, a large part of whose thesis consisted of very detailed histological descriptions of certain plants with reputed medicinal properties. The descriptions were good in the sense that the candidate had accurately recorded numerous histological details that he wished to convey. Furthermore, there were numerous excellent line drawings. The candidate had also adhered to the canons of good descriptive writing by working with carefully authenticated material and he had the intention of depositing a representative selection of his material in a well known herbarium collection of medicinal plants where it would be readily available to others. There was but one serious fault, namely that the descriptions were much too long. This was partly because the candidate had recorded a great many details which, with further experience, he would have realized were of little taxonomic significance. Secondly his descriptions were written in normal prose rather than in the botanical shorthand which I am here advocating. The following example will serve as an illustration. When describing the trichomes on the surface of the foliage leaves the candidate wrote as follows:—"Conical trichomes are present in large numbers on the interneural lamina but are absent over the veins: they show considerable variation in length and in their width at the base. The trichomes are uniseriate and project from either a unicellular or a multicellular and multiseriate dome-shaped base, which is raised above the general level of the epidermis". This passage has the great merit of being accurate and easy to follow; no exception can be taken to it as good descriptive writing in itself. But when we bear in mind that it was only one passage in a body of descriptive matter that filled a great number of typewritten pages, it will be appreciated that the style of writing was much too extended. The candidate could have described the trichomes on the leaf in some such way as this. Uniseriate, conical trichomes numerous, variable in length and in the width of their bases; restricted to interneural regions and arising from unicellular or multicellular, dome-shaped bases. This shorthand version requires only 27 words as opposed to the 57 written by the candidate. If he had adopted a similar style for all of the purely descriptive parts of his thesis it would have been much shorter and its quality much improved.

IS PUBLICATION IN PRINTED JOURNALS ALWAYS NECESSARY

In the course of descriptive studies it is inevitable that factual information will be accumulated which is not, in itself, of sufficiently wide interest to justify the the expense of having it printed and published in a journal. Nevertheless this same information could be of very great value to another worker or to a small group of workers in the same subject if some less expensive means could be devised for letting those who could use the information know that it exists. This is a problem that merits our serious attention at this moment when more articles are being published than it is possible to read. I have sometimes been approached by plant anatomists about the possibility of publishing their results in a journal, only to have felt that it would have been far more useful to circulate the information to a relatively small number, say 200 or so, of research workers who were actively concerned with the same or related subjects. What seems to be needed is the circulation of news bulletins rather than journals in which attention could be briefly drawn to work that is in progress. The detailed results of the various investigations might then be made available in the form of mimeographed circulars which could be obtained on application to the authors, or arrangements might be made for the circulars to be distributed through a recognized centre for the subject in question. Plant anatomy is a good example of a subject that provides scope for this method of data circulation. This procedure, in a sense, is equivalent to the present practice of circulating reprints, but it could be done at much less expense because the cost of printing the article in full in a journal would not have to be met, and to produce a mimeographed circular would cost much less. If subject matter that is ephemeral or of real interest to only a limited number of research workers could be distributed in this way, it would leave more space in journals for subject matter with a wider appeal or of more permanent value. Meanwhile the exchange of mimeographed articles might well lead to the publication of reviews, broad surveys or key works which would be of much greater scientific value than the mimeographed articles themselves. To adopt a scheme of this kind would involve making a change in our traditional approach to the publication of results, but it seems to me that we shall soon be driven to make a change by the ever increasing volume of subject matter that has to be handled. In recent years a series of mimeographed articles on plant anatomy has been prepared at Kew in the way that has just been suggested and circulated under the title of 'Notes from the Jodrell Laboratory'. The response has been very rewarding.

CONCLUSION

It is very obvious to us all that we are living in an age in which many changes are taking place. This then is a good moment to ask ourselves whether our traditional methods of communicating the descriptive aspects of biological science are adequate for our present needs.

I hope I have made it clear in this article that there will continue to be a great and important future for printed articles and books provided we adopt a selective attitude about their content. There can however, be little doubt that the present tendency to rush into print has now assumed such large proportions that it is outstripping its usefulness, and much of it is uneconomic. We must, therefore, consider alternative techniques, particularly for handling detailed factual information which is in itself dull, but nevertheless invaluable as source material for descriptive writing with a wider appeal.

These changes will need to be accompanied by an alteration in our psychological approach to our investigations. In these days we must recognize that any discipline that requires the accumulation of numerous factual data is more likely to progress through co-operative endeavours than through investigations undertaken by isolated research workers. Botanists engaged in collective research will need to appreciate that progress made by the co-operating group as a whole matters more than the personal prestige of individual members of the group. The individual will be rewarded by seeing the subject develop more effectively and by knowing that he is personally responsible for some part of the collective edifice of knowledge that the group is building.

In conclusion it will do no harm to remind oursleves that we all have a social responsibility when using our scientific knowledge. It follows that when any branch of knowledge can be used for the material as well as for the intellectual advantage of mankind, we should not fail to see that this is done. Besides attempting to solve fundamental problems, we should also remember that descriptive writers can place their knowledge at the disposal of all who have to deal with plants of economic importance. Judging from the popularity amongst young people of courses of instruction concerning economic plants at the present time, one cannot but feel that this is a direction in which descriptive histological work could be more effectively applied. Whenever we have an opportunity to describe the histology of plants that are actually or potentially useful we should devote special attention to them.

REFERENCES

HALSTEAD, L. B. 1971. Ph. D. Theses. *Nature, Lond.* **229**: 579.

WASHINGTON CORRESPONDENT. 1970 Demise of scientific journals. *Nature, Lond.* **228**: 1025-1026.

Adv. Pl. Morph. 1972: 85-91

FLORAL ANATOMY OF *RICINOCARPUS PINIFOLIUS* DESF. WITH SOME OBSERVATIONS ON THE PHYLOGENY AND CENTRE OF ORIGIN OF EUPHORBIACEAE

BY C. VENKATA RAO

Department of Botany, Andhra University Postgraduate Centre, Guntur, India

ABSTRACT

The floral anatomy of *Ricinocarpus pinifolius* (Stenolobeae), a Tasmanian member of Euphorbiaceae is described and the structure is compared with that of *Jatropha* (Platylobeae). The floral structure and trends of evolution in genera of the two sections of the Euphorbiaceae are compared and their origin from common geranialean stocks suggested.

The Euphorbiaceae are believed to have originated monophyletically on a southern land mass (Pangaea or Gondwanaland). The discontinuous distribution of the family mainly in southern continents and islands is attributed to continental drift.

INTRODUCTION

Pax and Hoffman (1931) divide the Euphorbiaceae into two sections, the Platylobeae (two sub-families Phyllanthoideae and Crotonoideae) with cotyledons much broader than the radicle and the Stenolobeae (Porantheroideae and Ricinocarpoideae) with cotyledons as narrow as the radicle. Though the nature of cotyledons is not a sound taxonomic criterion, several botanists (Willis, 1948; Rendle, 1952) have accepted this classification since the two sections of the family show distinct geographic distribution: while the Platylobeae are world wide in distribution, the Stenolobeae are exclucively Australian with the monotypic genus *Dysopsis* endemic in Juan Fernandes and Andes (Willis, 1948).

PREVIOUS WORK

There is no cytological, embryological or anatomical work in stenolobeae. The writer studied the floral anatomy of some members of Platylobeae and formulated the trends of evolution (Venkata Rao and Ramalakshmi, 1968). With a view to make a comparative study of these aspects in both sections the writer took up the study of the floral anatomy of *Ricinocarpus pinifolius*. In the light of the findings, an attempt is made in fixing the centre of origin of the Euphorbiaceae.

MATERIAL AND METHODS

Flowering material of *R. pinifolius* fixed in formalin-acetic-alcohol was kindly supplied by Dr W. M. Curtis, Hobart. This was dehydrated in alcohol series, cleared in xylol and infiltrated with para-

---

I am thankful to Dr W. M. Curtis, Department of Botany, Tasmanian University, Hobart for the fixed material of *Ricinocarpus pinifolius*.

ffin wax of 60°C m. p. Serial microtome sections were cut from 8-10 μ in thickness and stained in Delafield's haematoxylin.

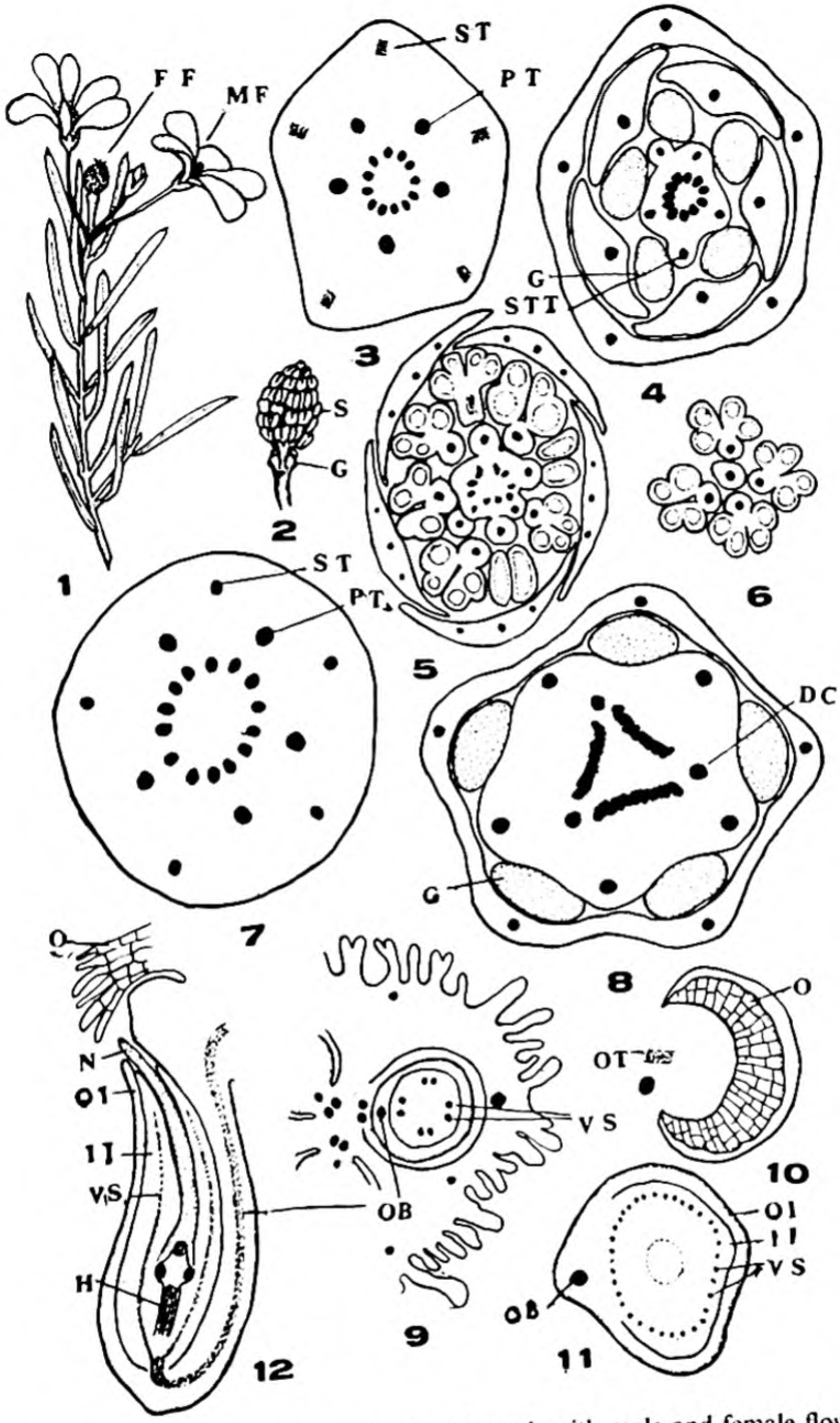
OBSERVATIONS

Ricinocarpus has 15 species in Australia (one extending into Tasmania) and one in New Zealand (Curtis, 1967). *R. pinifolius* is a shrub with clusters of 3-6 male flowers and one female flower occurring at the ends of branches (Fig. 1). The flowers are pedicellate (the pedicels of female flowers being much shorter and thicker than those of male), dichlamydeous, heterochlamydeous regular and 5-merous. The sepals are united at base; the petals are much larger, contorted and free to the base. The gynoecium is characteristically 3-carpellary, 3-locular with one ovule in each loculus; it is surmounted by 3 bifid styles. The male flowers show numerous stamens on a columnar androphore (Fig. 2). Both kinds of flowers have 5 thick ovoid alternipetalous glands (Figs. 2, 4, 8).

The thalamus in both kinds of flowers shows a ring of several discrete bundles from which ten traces depart in two alternating whorls for sepals and petals (Figs. 3, 7). Each trace divides into 3 bundles above the base of the respective organ (Fig. 5). Higher up the petal boundles give off some strands into the laminae of petals. So the parianth parts are 1-traced and 3-bundled as was also noticed in *Jatropha* (Venkata Rao and Ramalakshmi, 1968). This type of vasculature is considered to be very primitive (Puri. 1961). Five large, ovoid, non-vascular glands made up of richly protoplasmic cells stand alternate to the bases of the petals (Figs., 4, 8), as in *Jatropha glandulifera* and *J. curcas* (Venkata Rao and Ramalakshmi, 1968). A little above the emergence of petal traces five staminal traces are given off on the petal radii (Fig. 4). Thus the normal alternation of floral organs is interfered with and a sort of obdiplostemony is introduced due to the presence of glands as was also noticed in species of *Jatropha.* The numerous stamens of *R. pinifolius* seem to have resulted from the chorosis in both whorls of an ancestral obdiplostemonous androecium as was noticed in *Homonoia* (Venkata Rao and Ramalakshmi, 1968). However, the emergence of the staminal traces in regularly alternating pentamerous series is obscured due to the overcrowding. At the top of the column the whole of the stele is used up in the formation of staminal traces, there being no pistillode (Figs. 5, 6).

The stele of the female flower becomes triangular after the emergence of the perianth traces. A dorsal carpellary trace is given off from each of the angles (Fig. 8). This extends to the top of the ovary giving off branches in a pinnate manner into the thick ovary wall. The three arcs of vascular tissue found in the axial region of the ovary divide into two bundles each. One bundle from the adjacent pairs bends and both bundles enter into the base of the septum which becomes demarcated at this level. At the top of the ovary, one bundle from each pair enters into the funicle of the ovule (Fig. 10) while the other continues its course, fuses with the dorsal bundle and extends into one stylar branch. Thus anatomical studies show that the second ovule and its vascular strand are completely suppressed as was noticed in *Jatropha.*

The ovular bundle traverses downwards along the raphae and enters the chalaza. Here it divides into four main strands which bend upwards. Each bundle bifurcates and the four pairs of strands



FIGS. 1-12. *Ricinocarpus pinifolius.* Fig. 1. A branch with male and female flowers, x 1½. Fig. 2. Androecium and nectary of male flower, x 8. Figs. 3-6. Anatomy of male flower, x 18. Fig. 3. T. s. base of thalamus with sepal and petal traces. Figs 4, 5. T. s. flower bud at two levels. Fig. 6. T. s. top of the androecium. Figs. 7-11. Anatomy of female flower and ovule, x 18. Fig. 7. T. s. base of thalamus showing sepal and petal traces. Fig. 8. T. s. base of flower bud. Fig. 9. T. s. top of loculus showing placental obturator and emergence of ovular trace. Fig. 10. T. s. loculus with ovule; note four pairs of vascular strands in the inner integument. Fig. 11. T. s. ovule showing ring of vascular strands in the inner integument. Figs. 10, 11, x 38. Fig. 12. L. s. ovules with placental obturator, x 38.

FF, female flower; MF, male flower; ST, sepal trace; PT, petal trace; G, gland; STT, staminal trace; DCT, dorsal carpellary trace; O, obturator; OB-ovular bundle; VS, vascular strands; OI, outer integument; II, inner integument; N, nucellus: H, Hypostase.

traverse to about the middle of the height of the ovule (Fig. 9). Here they divide and form a ring of small strands which extends nearly to the top of the ovule (Figs. 11, 12).

The ovules are bitegmic, crassinucellar and slightly bent. The nucellus is narrow and cylindrical and extends as beak through the elongated micropyle which is formed by both the integuments. It nearly touches the placental obturator (Fig. 12). The outer integument is 4-5 layered and the cells are nearly empty; a few show tannin. The inner integument is massive and made up of richly protoplasmic cells. In morphological and anatomical features the ovules of *Ricinocarpus* resemble those of Platylobeae (Nair and Maitreyi, 1962; Nair and Abraham, 1963).

DISCUSSION

In the light of observations on the floral anatomy of some Platylobeae (Venkata Rao and Ramalakshmi, 1968) and *Ricinocarpus* (present studies), an attempt is made here in: tracing the phylogeny of Euphorbiaceae, formulating the evolutionary trends and fixing the centre of origin of the family.

Two views have been expressed regarding the phylogeny of Euphorbiaceae: (a) that they are derived from Sterculiaceae-like ancestors (Mitra, 1964; Hutchinson, 1968) and (b) that they are derived from geranialean ancestors (Venkata Rao and Ramalakshmi, 1968). The suggestion that the Euphorbiaceae are derived from Sterculiaceae-like ancestors seems to be untenable since the flowers in this family (as well as those of all other Malvales) are devoid of glands. The writer (Venkata Rao, 1952) suggested that the Malvales themselves were derived from Geraniales along a separate line in which the glands were lost early in phylogenetic history. The occurrence of pentacyclic, pentamerous and hermaphrodite flowers as well as apetalous, unisexual ones with vestigial sex organs in genera of Sterculiaceae shows that evolution within the family among the Malvales progressed parallel with that of Euphorbiaceae among Euphorbiales.

The occurrence of complete, bisexual, pentamerous, glanded flowers with obdiplostemonous androecium *regularly* at the lower dichotomies of the inflorescence in *Jatropha curcas* led Venkata Rao and Ramalakshmi (1968) to suggest that the Euphorbiaceae are derived from geranialean ancestors. Though the gynoecium in most Euphorbiaceae is tricarpellary, in *Wielandia* the monotypic endemic of Seychelles Is. it is 5-carpellary and forms a perfect connecting link between Euphorbiaceae and Geraniales. From this ancestral stock Euphorbiaceae evolved mainly by reduction which progressed synchronously and consistently in vegetative and reproductive parts as no where else among angiosperms including the two cactoid families Asclepiadaceae and Cactaceae.

Several features especially of the gynoecium of Euphorbiaceae, viz. the tricarpellary syncarpous pistil with 3 styles and 6 stigmas, anatropous pendulous ovules with ventral raphe, massive inner integument with vascular supply, elongated micropyle formed by both integuments, hairy placental obturator, beak-like nucellus extending to the obturator, caruncled endospermic seed and the fruit which is a schizocarp-capsule are so uniform and characteristic that a monophyletic origin is suggested for the family. Since it is unlikely that an ancestral stock with so many common characters could have originated at more than one time and in more than one place, monochrony and monotopy are also suggested.

The flowers in *Jatropha* (Platylobeae) and *Ricinocarpus* (Stenolobeae) are the most elaborate and therefore the most primitive in the respective sections and resemble each other in dichlamydeous heterochlamydeous perianth, well developed 1-traced, 3-bundled petals, non-vascular alternipetalous glands and obdiplostemonous plan of the androecium (though the flowers in *Ricinocarpus* have become unisexual due to reduction). These features point to the origin of the two stocks from common ancestors. A comparison of the floral structure in taxa of both sections (Table 1) shows that they evolved along parallel lines. However, evolution in Stenolobeae did not reach culmination as in Platylobeae; the cactoid habit, scale leaf, dwarf branch and cyathium noticed among the Platylobeae do not have their counterparts in the Stenolobeae. (*Euphorbia* and *Phyllanthus* reported from Australia and Tasmania are not indigenous but introductions of immigrants.)

Plant geographers like Cain (1944) have formulated certain criteria for fixing the centre of origin of a taxon, viz. species concentration, abundance of primitive life form (trees), occurrence of morphological, taxonomic and cytological primitives (diploids), endemism etc. These criteria proved useful in fixing the centre of origin of Proteaceae (Venkata Rao, 1967).

Perry (1943) found that polyploidy is of frequent occurrence in Euphorbiaceae and that their frequency in the family is about 50%. Neither are the diploids localised in distribution nor are the polyploids more wide ranging. Their distribution patterns seem to depend on their tolerance capacities and are not suggestive of centre of origin. So cytological data do not seem to be of so much use in fixing the centre of origin of Euphorbiaceae.

The Stenolobeae are exclusively Australian in distribution with one genus (*Dysopsis*) endemic in Andes and Juan Fernandez. A similar pattern of distribution is noticed in Epacridaceae in which all the 29 genera

TABLE I

No.	Floral character	Genus of Platylobeae	Genus of Stenolobeae
1.	Dichlamydeous, heterochlamydeous, 5-merous perianth	*Jatropha*	*Ricinocarpus*
2.	Dichlamydeous, 3-merous perianth	*Phyllanthus*	*Microntheum*
3.	Monochlamydeous, 5- or 3-membered perianth	*Manihot*	*Pseudenthus*
4.	Nectary of 5 alternipetalous lobes	*Jatropha*	*Ricinocarpus*
5.	Androecium basically obdiplostemonous	*Chrozophora*	*Ricinocarpus*
6.	Numerous stamens formed due to chorosis in both whorls	*Croton*	*Ricinocarpus*
7.	Stamens 6 or 3+3	*Tragea*	*Pseudanthus*
8.	Stamens 5 in one whorl	*Phyllanthus*	*Poranthera*
9.	Staminodes or pistillodes present	*Chrozophora*	*Micrantheum*
10.	Two collateral ovules in a carpel	*Breynia*	*Poranthera*

of the family are endemic in Australia and one genus (*Lebetanthus*) in Patagonia. S. America seems to have been connected with Australia via Antarctica and genera could have migrated via an antarctic land route. The Platylobeae show two main centres of distribution, tropics of Africa and Americas. (Lawrence, 1964). Two patterns of distribution are evident: (a) some genera are endemic in continents or adjacent land masses or even small islands and (b) some genera are common to two continents or adjacent land masses. These are strongly suggestive of the origin of the family on a southern land mass, Pangaea or Gondwanaland.

Trees are abundant in S. Africa and S. America (species of *Euphorbia, Toxicodendrum, Jatropha, Hevea, Manihot* etc.). Several of these taxa show most primitive morphological features viz., large compound or lobed leaves, dichasial cymes and perianthed (sometimes bisexual) flowers. Endemism is also high: *Gelonium, Bridelia, Ricinus* are endemic in Africa while a large number of genera (e. g., *Manihot, Hevea, Hippomane* etc.) are endemic in S. America and Brazil (Lawrence, 1964). Several cyathium bearing genera viz. *Elaeophorbia, Synadenium, Monadenium, Dichostemma* and *Stenadenium* are endemic in S. Africa while *Anthostema* has two species in S. Africa and one in Madagascar. The cyathia in these genera are of the actinomorphic (*Euphorbia*) type. The presence of perianth in the male flowers of *Anthostema* and *Dichostemma* shows that the cyathia are of the most primitive type and that probably the cyathium originated in this region. The presence of *Agaloma, Tithymalus, Poinsettia* and *Pedilanthus* with zygomorphic cyathia in the American region shows that the original stock received from Africa diversified and evolved to a greater extent.

Some taxa are common to two or more land masses: *Jatropha* is common to Africa and India; *Dalechampia* is common to Africa and S. America *Cluytia* is common to Africa and Arabia and *Hura* and *Euphorbiodendron* to S. America and W. Indies. *Caperomia* is common to Brazil, Central America, W. Indies and tropical Africa. These patterns of distribution are suggestive of their origin on a connected land mass and segregation due to continental drift. Some genera are endemic in small islands e.g., *Cubanthus* in Cuba, *Neogullauminia* in New Caledonia, *Wielandia* in Seychelles etc. Endemism suggested age and long period of isolation.

From the patterns of distribution described above, the following conclusion can be drawn; the family originated under tropical conditions on a once connected land mass Pangaea or Gondwanaland. When this broke up, the ancestral stock became distributed mainly among the three continents Australia, Africa and America, while a few taxa were isolated among some islands. Australia seems to have separated relatively earlier, by which time a primary differentiation took place into stocks ancestral to the Stenolobeae and Platylobeae. The Stenolobeae were mainly received by Australia while the Platylobeae were received by remaining part of the landmass. Though evolution progressed in Stenolobeae along lines essentially similar to those in Platylobeae, the geographical isolation and less scope for hybridization resulted in less diversification and absence of climax types. Under greater variety of ecological conditions and scope for hybridization, the Platylobeae diversified to a greater extent and resulted in a multitude of vegetative and floral types. The taxa with greater tolerance capacity spread far and wide and even extended into higher latitudes and altitudes, making the family almost cosmopolitan.

REFERENCES

CAIN, S. A. 1944. *Foundations of Plant Geography*. Harper. N. Y.

CURTIS, W. M. 1967. *Students' Flora of Tasmania*. Part 3. Hobart.

HUTCHINSON, J. 1968. Tribalism in the family Euphorbiaceae *Am. J. Bot.* **39**: 657-707.

LAWRENCE, G. M. L. 1964. *Text Book of Vascular Plants*. Oxford.

MITRA, J. M. 1964. *An Introduction to Systematic Botany and Ecology*. World Press. Calcutta.

NAIR, N. C., AND V. ABRAHAM. 1963. A contribution to the morphology and embryology of *Micrococca mercurialis*, *J. Indian. bot. Soc.* **42**: 483-493.

NAIR, N. C., AND M. MAITREYI. 1962. Morphology and embryology of *Sebastiania chamaleae*. *Bot. Gaz.* **124**: 158-168.

PAX. F., AND K. HOFFMAN. 1931. Euphorbiaceae. In *Die naturlichen Pflanzenfamilien* by A. Engler and K. Prantl. Leipzig.

PERRY, B. A. 1943. Chromosome numbers and phylogenetic relationships in the Euphorbiaceae. *Am. J. Bot.* **30**: 527-543.

PURI, V. 1951. The role of floral anatomy in the solution of morphological problems. *Bot. Rev.* **17**: 471-553.

RENDLE, A. B. 1952. *Classification of Flowering Plants*. Cambridge.

VENKATA RAO, C. 1952. Floral anatomy of some Malvales and its bearing on the affinities of the families included in the order. *J. Indian bot. Soc.* **31**: 171-243.

VENKATA RAO, C. 1967. Origin and spread of the Proteaceae. *Proc. natn. Inst. Sci. India.* **33B**: 99-108.

VENKATA RAO, C., AND T. RAMALAKSHMI. 1968. Floral anatomy of Euphorbiaceae-I. Some non-cyathium taxa. *J. Indian. bot. Soc.* **47**: 278-300.

WILLIS, J. C. 1948. *Dictionary of Flowering Plants and Ferns*. Cambridge.

Adv. Pl. Morph. 1972: 92-101

AN EXTENDED INVESTIGATION OF THE MEGAGAMETOPHYTE IN *OXALIS CORNICULATA* L.

By J. M. Hfrr, Jr.

University of South Carolina, Columbia, S. C., U. S. A.

Abstract

In this study, a new clearing technique is used in place of the standard paraffin section method to extend earlier investigations of the megagametophyte in *Oxalis corniculata* L. Attention is directed chiefly to the 4-nucleate stage and to the orientation of the egg apparatus in the mature megagametophyte. All observations are recorded from optical median sagittal sections of the hemianatropous ovule focussed either through the left or right side. Initially, the micropylar nuclei of the 4-nucleate stage lie on the transverse axis of the megagametophyte, while the chalazal nuclei are positioned on the longitudinal axis. The nuclei of the micropylar pair are designated as A and B according to their position away from or toward the funiculus respectively. In the course of ontogeny, the micropylar nuclei move from the transverse to the longitudinal axis, and either nucleus, A or B, shifts chalazally during the reorientation. Two orientation patterns for the egg apparatus previously reported are here confirmed. In most cases, the egg is situated towards the right side of the ovule with the synergids toward the left, or less frequently the opposite arrangement occurs, i.e., egg toward the left, synergids toward the right. In a third pattern, heretofore not reported, the egg is centered between the synergids which lie toward the left and right sides of the ovule. The synergids consistently curve towards the funiculus. Present information on nuclear migration and position of division figures cannot account for the latter orientation pattern of the egg apparatus, and an extension of this study is, therefore, required.

Introduction

From time to time, published reports are featured either by title or introductory comment as reinvestigations of topics studied in the past. In most cases, the author of the reinvestigation did not participate in the earlier study, a point usually forcibly clarified at the onset. Readers, accordingly, expect reinvestigations to represent the step by step retracing of one investigator's research by another with the revelation and correction of errors along the way. Indeed, many studies labelled as reinvestigations from initial design through implementation fulfill this concept. Often, however, such studies only tenuously encounter the methods and observations of some earlier student of the topic. Instead, the second investigation often employs an entirely different approach to broaden positively the concepts established earlier, and reinvestigation in the popular connotation of the term becomes a misnomer. Such studies would be more appropriately designated as continued or extended investigations. They should be recognized as especially valuable to those centres of inquiry currently influenced either by changing philosophical concepts or by advances in technology.

The megagametophyte of angiosperms, a subject of intense investigation for nearly a century, has been approached from a variety of philosophical bases and accordingly subjected to numerous interpretations. Nevertheless, that the megagametophyte has evolved through reduction from a level of complex structural organization to its present simple form certainly emerges as a point of general agreement. The severity of phylogenetic reduction seemingly has imposed an aspect of uniformity upon megagametophytes which has received an emphasized attention in many investigations. This emphasis is especially reflected in such published statements here paraphrased as, "Seventy per cent of the angiosperms display the normal or Polygonum type megagametophyte......" or, "The species in question follows the Polygonum type of development with usual, 2, 4, and 8-nucleate stages followed by normal organization of the mature embryo sac." Fortunately, however, several investigators of prominence have pointed out the wide diversity displayed by the megagametophyte throughout angiosperms, and among these Schnarf (1929) and Maheshwari (1950) stand pre-eminent. The broad variations noted especially by these two investigators considered with other embryological data are useful in the definition of the major taxa within angiosperms. Indeed, in many cases, too numerous to cite here, the megagametophyte has proved valuable in settling the issue of correct affinity for families and genera.

The unique character of the megagametophyte in a particular species is expressed over its entire ontogeny through subtle and often obscure features of development. The obscurity of these features, which collectively would accord species identity to the megagametophyte, originates from two sources—the overwhelming uniformity of structure in the megagametophytes of any two species, the natural consequence of phylogenetic reduction, and the absence of a technology which would permit a thorough monitoring of ontogeny. Thus, so obvious is the similarity between two species of megagametophytes both of which follow Polygonum type development that an occasionally observed difference between them which involves the division plane of the micropylar nucleus of the 2-nucleate stage seems insignificant. However, such a difference as a nuclear division plane monitored sufficiently to establish it as the rule rather than the exception should not be overlooked. In the absence of a complete reiteration, attention is here directed to an applicable hypothesis stated earlier (Herr, 1967) that the quality of a specific variation between two morphological entities (morpha), its magnitude as an expression of diversity, is inversely proportional to the structural complexity of the two morpha. By this tenet, two comparable morpha such as the 2-nucleate megagametophytes of two species are in structural organization very simple as a result of phylogenetic reduction and incomplete ontogeny as well. Therefore, a consistent difference between these megagametophytes which involves the division plane of the micropylar nucleus in each one constitutes a variation of great magnitude and hence significance. That the reduction of structural and organizational complexity, manifested in the megagametophyte of angiosperms, has not imposed a reduction in potential variation is the logical conclusion of this theme. The reality of variation, however, cannot be disclosed if the small points of distinction are relegated to obscurity.

The thesis is here proposed that the megagametophytes of all angiosperms previously studied should be subjected to extended investigations, studies which emerge from and enlarge upon the valid discoveries of the past. Such an investigation is presented here for *Oxalis corniculata* L. previously investigated by Hammond (1908) and by Herr and Dowd (1968). Megagametophyte development in Oxalidaceae, as is the case for many families, has been initially explored in only a few taxa. Among these, *O. corniculata* shows several strikingly unusual features which should be accorded further study. For example, cytokinesis following meiosis I is often concomitant with the organization of the daughter nuclei or with equal frequency is delayed until the daughter nuclei are fully established. Megagametogenesis follows either the Allium bisporic type or less frequently the Polygonum monosporic type. The orientation of the egg apparatus is an especially unusual feature and represents an unexpected consequence of development from the 4-nucleate stage as it is presently understood. In median sagittal sections of the ovule, the synergids lie side by side either above or below the plane of the egg. Thus, two orientations of the egg apparatus occur, the egg towards the right side of the ovule and the synergids towards the left or vice versa. In the 4-nucleate megagametophyte, as it appears in median sagittal sections of the ovule, the micropylar nuclei lie side by side on the transverse axis, while the chalazal pair is positioned vertically on the longitudinal axis. None of the division planes possible for the micropylar nuclei so fixed onto the transverse axis would place the nuclei of the micropylar quartet in a position from which the two known orientation patterns of the egg apparatus could be derived. Obviously a shift in the position of the micropylar nuclei during either the 4- or 8-nucleate stage must occur, and to the question inherent in his conclusion is this extended investigation directed.

Throughout this century, the paraffin section technique has provided the principal tool for embryological investigation. The method is indeed arduous, time consuming, and hence somewhat inadequate for a close monitoring of finite ontogeny. To examine such features as the position of nuclei and orientation of specific cells requires the accurate selection of precise section planes which in practice is difficult to achieve. Therefore, this standard method has been abandoned in this study and replaced by a recently developed clearing-squash technique described by Herr (1971). The new technique, especially suited to a study of this kind, has already undergone certain modifications which are here described.

MATERIAL AND METHODS

Expanded flower buds in the final stage of development prior to anthesis were collected from a small population of *Oxalis corniculata* maintained in the greenhouse since 1964. Voucher specimens for this collection are on deposit in the University of South Carolina herbarium. The pistils were removed from the flowers, fixed for twenty-four hours in FPA 50 (formalin, propionic acid, 50% ethanol, 5:5:90), and stored in 70% ethanol. Initially, the ovules were dissected from the pistils and transferred to a recently developed clearing fluid composed of lactic acid (85%), chloral hydrate, phenol, clove oil, and xylene (2:2:2:2:1, by weight). After treatment for twenty-four hours at room temperature, the ovules appeared nearly transparent under a Wild M-5 dissecting microscope equipped with transmitted light. By means of a pasteur pipette, they were transferred with a small amount of clearing fluid to special slides for microscopic examination. These slides are similar to the micro-culture units used in studies of isolated protoplasts (Raj and Herr, 1970). Two cover glasses (No. 0-2, 12 or 18 mm sq.) were affixed with permount or balsam, 1 cm apart on a microscope slide which then was placed on a warming table at 50° C for three days to insure adequate hardening of the mounting medium. A drop of clearing fluid with several ovules was placed in the centre of the 1 cm space, and a cover glass (No. 0, 12 or 18 mm sq.) was placed over the preparation so to rest on the two mounted cover glasses. The support cover glasses must be of sufficient thickness to eliminate pressure by the top cover on the ovules. All preparations were examined under the phase contrast optics of a Wild M-20 microscope. The unique action of the clearing fluid permitted the study of cellular structure with the phase oil immersion objective focused at any focal plane within the ovule.

The hemianatropous ovules tend to lie sideways, that is, with the long axis of the megagametophyte parallel to the slide. Downward focusing, therefore, reveals ovule structure through a series of optical sagittal sections. If the ovule is slightly tilted so that the optical section is oblique, a slight adjustment of the cover glass on the support mounts positions the ovule precisely for the desired sagittal view. By a more pronounced shifting of the cover glass, ovules in the early stages of development, during megasporogenesis, can be reoriented for optical sections in both the frontal and transverse planes. Thus, a single ovule can be examined from sagittal, frontal, and cross sectional aspects.

After a brief exposure to the clearing fluid, the ovules become quite fragile. If, therefore, the cover glass is lightly and repeatedly pressed with a dissecting needle midway between the support covers, the cells of the ovule gradually become spread apart. This progressive squash procedure, here performed under observation with a Wild M-5 dissecting microscope, does not disrupt the structural integrity of the individual cells. Furthermore, it provides a method for extending the observations to the detailed features of specific structures such as the developing megagametophytes previously identified within intact ovules. Especially in *Oxalis*, a very slight application of the progressive squash procedure effects removal of the inner integument with the enclosed remnant nucellus and developing megagametophyte.

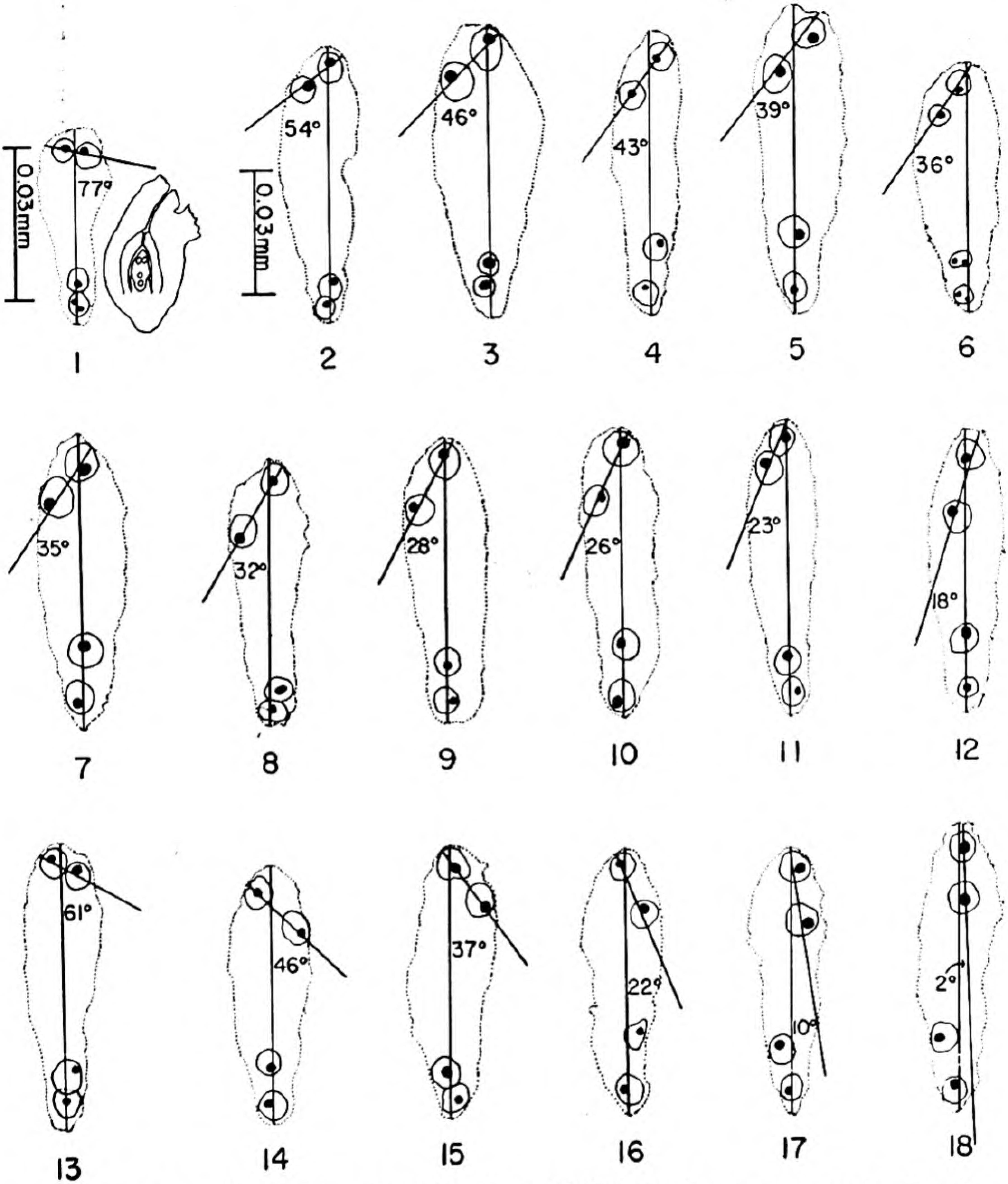
Later in this study, the clearing method was modified with decided improvement. The dissected ovules were first treated with 2% KOH dissolved in 70% ethanol, and then washed in several changes of 70% ethanol. They were then subjected to a mixture of 85% lactic acid and 70% ethanol (1:1) for twenty-four hours, dehydrated to absolute ethanol, and placed in the clearing fluid. Clearing fluid to which a small amount of iodine was added (100 mg iodine per 9 gms clearing fluid) proved more adequate than the original fluid and greatly enhanced the phase contrast image of the megagametophyte.

The drawings and photomicrographs were produced with the Wild Heerbrugg drawing tube and camera equipment respectively. Figure 24, a composite photograph, was prepared by combining the images depicted in figures 22 and 23. Pollaroid transparencies of figures 22 and 23 were mounted together as described by Smith and Herr (1971) for projection of the combined image on a screen. The combined image was then projected on negative film from which figure 24 was printed.

OBSERVATIONS AND DISCUSSION

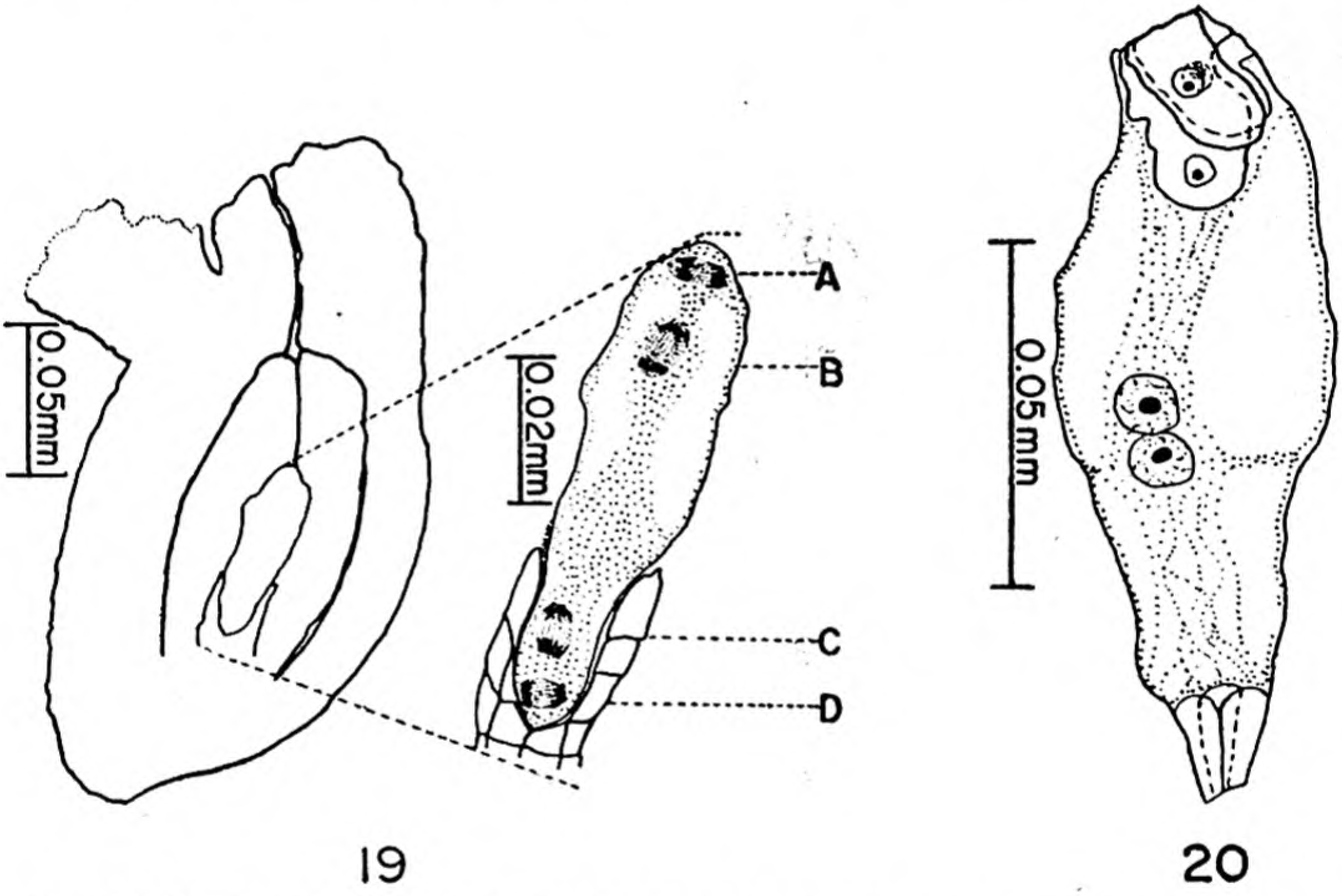
The previous report by Herr and Dowd (1968) that division of the two nuclei in the 2-nucleate megagametophyte follows a precise and consistent pattern has here been confirmed. The orientation of the division figures is best observed and described from the optical sagittal section of the ovule. From this aspect, the micropylar division figure is positioned on the transverse (horizontal) axis of the megagametophyte, while the chalazal division figure is placed on the longitudinal (vertical) axis. Thus, in the optical median sagittal section of the ovule, the micropylar nuclei of the 4-nucleate stage lie side by side on the transverse axis. The chalazal nuclei lie one above the other on the longitudinal axis. This arrangement allows for individual designation of the micropylar nuclei, for one nucleus is closer to the funiculus of the hemianatropous ovule than is the other. For later reference here, the nucleus away from the funiculus is designated as nucleus A and the one closest to the funiculus as nucleus B (Fig. 1). If a line is constructed through the geometric centre of each nucleus of the micropylar pair, the exact position of the nuclei can be expressed in terms of the angle formed by the constructed line and the longitudinal axis of the megagametophyte. The intersecting lines divide the megagametophyte into four sectors, two toward the micropyle and two toward the chalaza. By the description furnished here, one would expect these sectors to be quadrants i.e., each one with an angle of 90°, at initiation of the 4-nucleate stage. Actually, however, the angle of the chalazal sector on the funiculus side is either slightly less or greater than 90° indicating that the initial alignment of the micropylar nuclei on the transverse axis is only approximate. This angle in fig. 1, for example, is 77°. Nevertheless, to the observer the alignment of the nuclei appears as approximately transverse (rather than oblique) when this angle measures between 75° and 105°.

A close monitoring of the 4-nucleate stage, achieved by the examination of many examples, reveals a distinct shift in the position of the micropylar nuclei from the transverse to the longitudinal axis. The reorentiation is accomplished by a pronounced chalazal shift of either nucleus A or nucleus B. In most cases, actually in 40 of 60 examples studied, nucleus A shifts its position chalazally, and the angle of the chalazal sector away from the funiculus is especially useful for the monitoring of this shift. The angle decreases as the nuclei become closer aligned to the longitudinal axis (Figs. 2-12). Less frequently (20 of 60 examples), nucleus B moves chalazally, and the shift is expressed as a decrease in the angle of the chalazal sector toward the funiculus (Figs. 13-17). The end result in either case is the vertical alignment of the nuclei (Fig. 18). In examples where the reorientation to the vertical alignment has been achieved, determination as to which nucleus, A or B, makes the pronounced shift is not possible. Accordingly, in fig. 18, nucleus A may have moved 2° passed the vertical. On



FIGS. 1—18. Reorientation of the micropylar nuclei of the 4-nucleate megagametophyte from the transverse to the longitudinal axis of the ovule. All figures drawn from optical sagittal sections through the right side of cleared ovules. Fig. 1. Micropylar nuclei aligned approximately on the transverse axis (77° from the longitudinal axis). Nuclei A and B positioned respectively away from the axis toward the funicular attachment. Figs. 2-12. Progressive chalazal shift of nucleus A from an obliquely transverse axis (54°) to an approximately longitudinal axis (18°). Figs. 13-17. Progressive chalazal shift of nucleus B from an obliquely transverse axis (61°) to an approximately longitudinal axis (10°). Fig. 18. Micropylar nuclei aligned approximately on the longitudinal axis (2°). Alignment attained through chalazal shift of either nucleus.

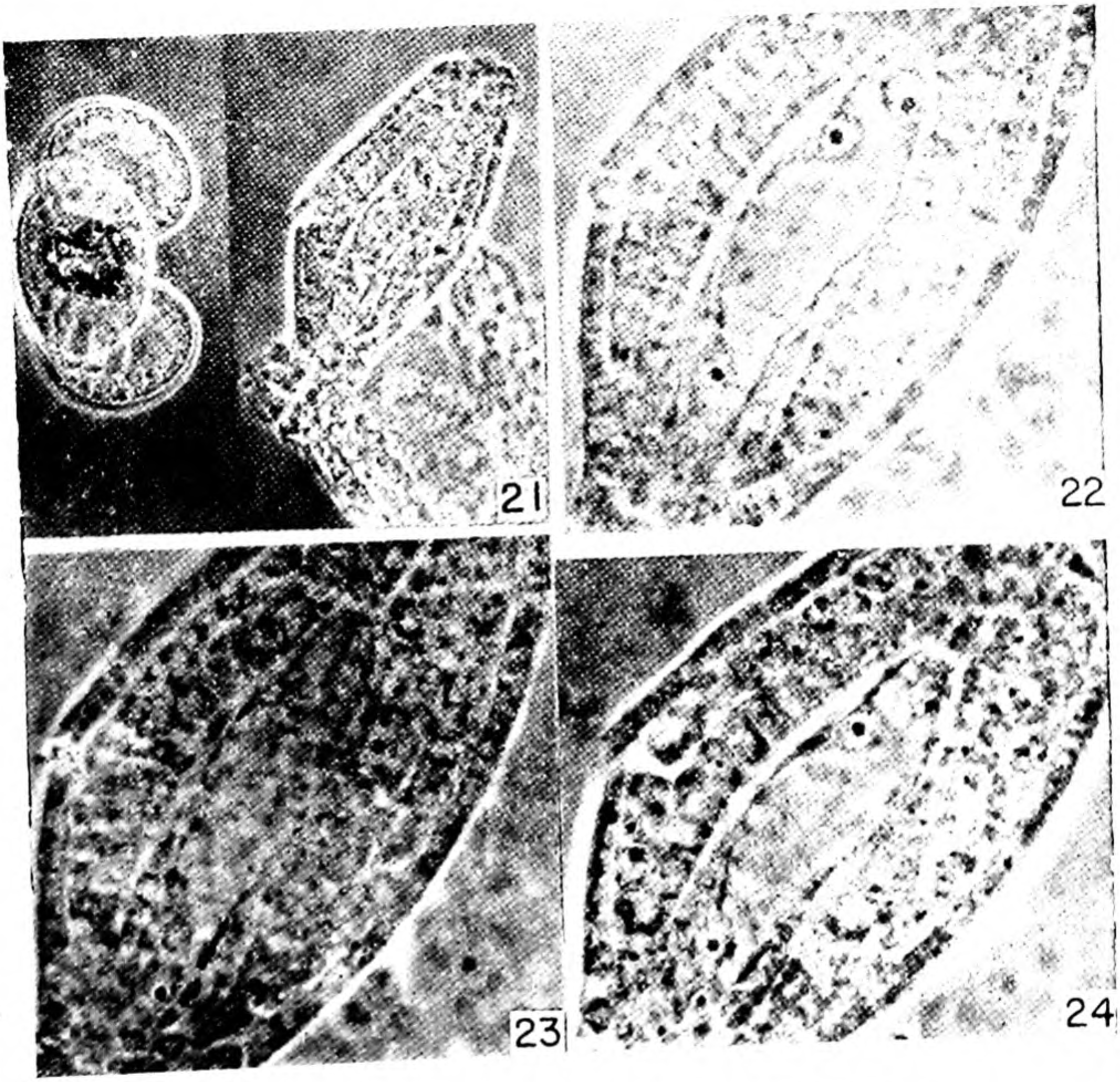
the other hand, nucleus B may have moved to a position only 2° away from the longitudinal axis.



FIGS. 19, 20. Fig. 19. Nuclear divisions in the 4-nucleate megagametophyte from optical sagittal section through left side of cleared ovule. Mitotic figures A and C in upper focal plane toward left side of the ovule; B and D in lower focal plane toward right side of the ovule. Fig. 20. Mature megagametophyte from optical sagittal section through right side of cleared ovule. Egg centered between two synergids; synergids adjacent to right and left sides of the ovule.

In truly median optical sagittal sections of the ovule, the micropylar nuclei on the transverse axis lie in the same focal plane (Fig. 1). With reorientation to the longitudinal axis, the shifting nucleus, A or B, becomes positioned either above or below the focal plane of the other one. The final mitotic division in the megagametophyte, not yet monitored sufficiently, closely follows the alignment of the micropylar nuclei to the logitudinal axis. In many cases, the division figure of the micropylar nucleus crosses the transverse axis while the chalazal figure (nucleus A or B) is positioned on the longitudinal axis (Fig. 19). In figure 19, from an optical median sagittal section through the left side of the ovule, the division figures are arbitrarily designated as A and B. Division figure A lies in the upper focal plane, while figure B is positioned in the lower focal plane. Therefore, without a subsequent shift in position by the daughter nuclei of these divisions, the synergids (from figure A) would lie toward the left side of the ovule, while the egg and micropylar nucleus would be positioned towards the right side. The position of the mitotic spindles in figures 19 correlates with the most common orientation pattern of the egg apparatus, a feature to be discussed later.

Although the 4-nucleate megagametophyte is clearly recognizable in cleared, intact ovules, it can be studied to better advantage if enclosed only by the inner integument. A careful application of the progressive squash technique permits removal of the inner integument and enclosed megagametophyte without altering its position in relation to the geometry of the intact ovule. Thus, with the ovule situated for observation from its right side, the inner integument can be



FIGS. 21-24. Inner integument and enclosed 4-nucleate megagametophyte removed from ovule by progressive squash technique. Fig. 21. Inner integument and enclosed 4-nucleate megagametophyte emerging from micropylar region of squashed ovule viewed from the right side. Three nuclei in megagametophyte in optical sagittal section selected. Pine pollen grain (left) provides apt measure to emphasize the minute size of the 4-nucleate stage, x 414. Fig. 22. Optical sagittal section through inner integument and 4-nucleate megagametophyte of figures 21. One chalazal and two micropylar nuclei visible, x 1020. Fig. 23. 4-nucleate megagametophyte of figure 22; optical sagittal section reveals second chalazal nucleus, x 1020. Fig. 24. Composite photograph of figures 22 and 23. All four nuclei of megagametophyte visible; nucleus A of micropylar pair in chalazal shift towards longitudinal axis of ovule, x 1020.

Adv. Pl. Morph. 1972: 102-112

MORPHOLOGY AND EMBRYOLOGY OF THE FAMILY CYPERACEAE—AN APPRAISAL

BY C. K. SHAH

Botany department, Gujarat University, Ahmedabad-9, India

ABSTRACT

This paper presents an analysis of the main events in the reproductive cycle of sedges. It also assesses the value of the embryological findings with respect to the affinities of the family Cyperaceae.

The embryological data together with morphological and anatomical findings indicate that the family Cyperaceae shows definite relationships with the Juncaceae. Affinities with the Gramineae are doubtful and its consideration is rejected from all the sources.

The Cyperaceae exhibit a combination of such morphological and embryological features which are, as a rule, not common to the family Gramineae with which it was previously associated. In view of this, Hutchinson's (1969) and Takhtajan's (1969) suggestions that the Cyperaceae should be raised to the rank of an order and be designated 'Cyperales' in the frame work appears to be reasonable.

INTRODUCTION

Many investigators have questioned the traditional Cyperaceae-Gramineae alliance and have proposed a relationship of Cyperaceae with Juncaceae instead. So marked are the resemblances between some members of the Cyperaceae and certain species of *Juncus* and *Luzula* that these two presumably form an intermediate stock between the families Liliaceae and Cyperaceae (Thorne, 1963). On the other hand the genus *Oreobolus*, a primitive genus of the Cyperaceae is very closely related to the Juncaceae in bearing a distinct perianth of six segments (Takhtajan, 1959; Kubitzki, 1966) but it has not yet received an embryological study (Maheshwari, 1964). The stamens in the order Pandanales have a nearly sterile apex of the connective. This feature is common to the Cyperaceae and may possibly serve as a link between Cyperaceae and Pandanales (Holttum, 1948). Following Holttum, Kern (1962) regards the derivation of the Juncaceae from Liliflorae as impossible. Lawrence (1951) has gone a step further and suggested that even the three subfamilies of Cyperaceae (Scirpoideae, Rhynchosporoideae, Caricoideae) should be treated as independent families: Cyperaceae (restricted to the sub-family Scirpoideae), Rhynchosporaceae and Caricaceae. The problem is not whether Cyperaceae are more closely related to Juncaceae than to Gramineae; there is almost unanimous agreement that Cyperaceae and Gramineae are not intimately allied (Shah, 1967). The main questions are : Can Cyperaceae be derived from Liliales especially Juncaceae ? What

---

* The author is grateful to the Gujarat University for financing the study.

has been done mainly on the alleged primitiveness of the genus *Oreobolus* with its perianth so similar to that of Juncaceae? And are the differences within Cyperaceae so essential that the family should be split up into 2 or 3 smaller families or is it a natural unit? The following account was undertaken to determine what light could embryological findings throw on the taxonomic treatment of this family.

ANTHER AND MALE GAMETOPHYTE

Each anther consists of four sporangia. Rarely two sporangia belonging to different thecae fuse in *Cyperus articulatus* (Shah, 1962a) resulting in a trilocular condition (Khanna, 1965). It has a persistent epidermis of enlarged or papillate cells, the endothecium develops fibrous thickenings (exothecium in *Chemaegyne*) (Süessenguth, 1954), 1 or 2 middle layers are ephemeral and the cells of the glandular tapetum may become binucleate exhibiting ubisch granules on their inner surfaces. The acute apices of the wedge-shaped microspore mother cells face the centre of the cylinder (Carniel, 1962). The wall of the microspore mother cell does not continue as exine but a mucilaginous wall is secreted between its protoplast and cell wall (Lewis and John, 1961). After meiosis II, all the four nuclei arranged in a tetrahedral, decussate or isobilateral manner lie free in the cytoplasm.

All the microspores of an anther undergo contraction. It appears as if all the grains have set in degeneration. This shrinkage lasts up to the formation of bicelled grain. It causes temporary shrinkage which makes the young pollen grain look abortive (Shah, 1962c). Then the grain gradually expands but the inner cytoplasm remains contracted. The grain has larger concavities on the sides and a smaller one at the distal end. Thus the tetranucleate cells assume a variety of forms due to a wave of contraction stamping irregular density of cytoplasm. During the division of the generative cell, the grain assumes its ovoidal form. The concavities are eliminated but their imprints remain on the cytoplasm (Shah, 1967) and appear as folds on the exine (Padhye, 1966). The special orientation of the tetrad nuclei in relation to the narrow proximal part of the pollen grain depends on contractions in the cytoplasm (Shah, 1960, 1962).

The three sterile nuclei occupy the apex of the wedge in all species (Shah, 1962a) except *Rhyncospora japonica* (Tanaka, 1941) where they move to the wall adjacent to the tapetum. The exceptional case of *Rhyncospora* is illuminating because it shows that the physiological gradient in the PMC and the grain is not relative to the tapetum—the source of supply—but to the centre of the cell (Lewis and John, 1961).

After meiosis, the four chromosome plates of the tetrad are not fully disorganised. One of them grows and after a short time passes into first pollen mitosis. The chromosomes of the other three nuclei soon show signs of degeneration but may pass into a stage similar to the metaphase in *Eleocharis*, sub. ser. *palustris* (Strandhede, 1965) or even organise small nuclei as in *Carex wallichiana* (Shah, 1962a). The chromosomes in the non-functional nuclei are smaller than those in the favoured nucleus. This may reflect a difference in auto- and/or hetero-catalysis (Lewis and John, 1961).

In the majority of angiosperms, the microspore nucleus comes to lie at one end of the cell due to the formation of a large vacuole. In Cyperaceae no vacuolation is seen in the microspores. Both asymmetrical and symmetrical spindles have been observed during the divisions of the functional nucleus. A cell plate laid down between the two nuclei rests upon the septum separating the vegetative from the 3 effective nuclei. The generative cell rests upon the septum which separates the three effective nuclei. Although some of Piech's (1924, 1928) figures show a generative cell lying free in the cytoplasm, his other figure clearly indicates that the generative cell rests on the plasma membrane. Similarly Khanna's (1965) contention of a free cell formation around the generative nucleus is not corroborated by her figures.

When the 3 sterile nuclei are thrust at the apex of the wedge-shaped cells, they are delimited by a hyaline plasma membrane and further separated from each other by thin membrane and have discrete protoplasts around the sterile nuclei. Thus cytokinesis is simultaneous but extremely delayed. The formation of membranes between the individual nuclei is variable but in some instances where they are ephemeral, the membranes may have been overlooked. Abortive divisions in 3 effete nuclei form 5 or 6 micro-nuclei. Their remnants lie adpressed to the intine.

In *Eleocharis* (Strandhede, 1965) gametes with aberrent chromosome numbers or with aberrent lengths of single chromosomes are morphologically well developed and obviously viable. The pseudomonad is 3-celled, monocolpate with an ulceroid aperture on the side opposite to 3 germinating nuclei. The above sequence of events leads to the well known divergent nature of sedge pollen.*

OVULE AND FEMALE GAMETOPHYTE

There is a single basal anatropous ovule in the ovarian chamber. In *Bulbostylis barbata* unicellular hairs arise from the funiculus and approach the micropyle. These form a loose type of an obturator. But in *Carex wallichiana* and *Cyperus articulatus*, the cells of the funiculus divide and form a compact obturator (Shah, 1959; Khanna, 1963). Their cells are richly cytoplasmic and grow to form a canopy covering the micropyle. They wither after fertilization and probably provide nourishment to the pollen tubes. Earlier workers have missed the obturator. Some of the nucellar cells towards the chalazal region of the embryo-sac become cutinised. The cells of this padlike tissue become enlarged and filled with tannin-like substance resulting in the formation of a hypostase.

The development of the embryo sac conforms to the Polygonum type as has also been reported in *Carex capricornis*, *C. makinoensis*, *C. marrowii* (Tanaka, 1950), *C. aristata* (Grezicka, 1964), *Cyperus rotundus*, *Scirpus mucrinatus*, *Kyllinga melanospora* (Khanna, 1965), *Fimbristylis quinquangularis* (Dnyansagar and Tiwari, 1956), *F. tenera* (Gupta, 1959, 1962), *F. diphylla* (Murty and Kumar, 1967) and *Kyllinga triceps* (Gupta, 1959).

EMBRYO

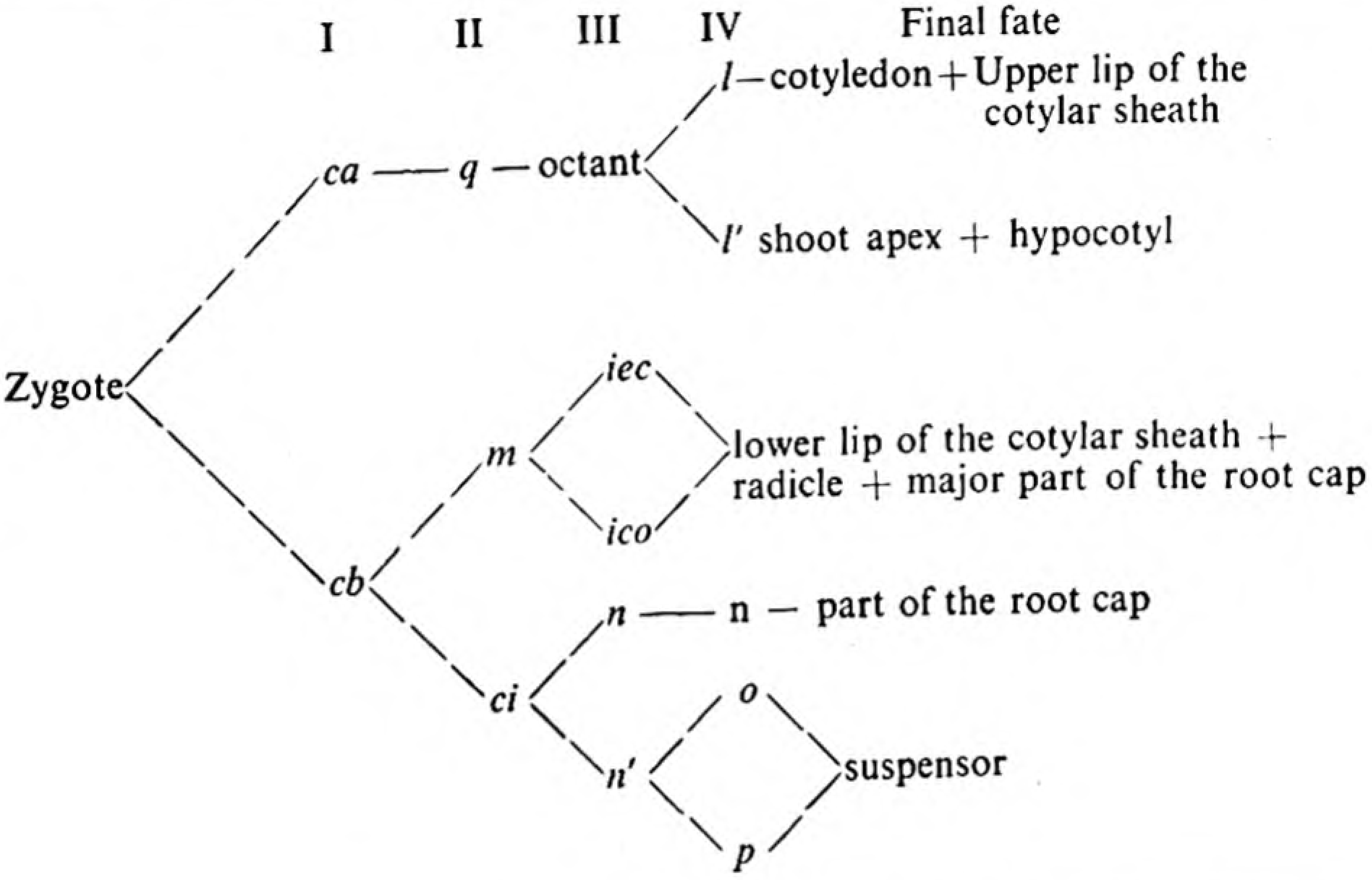
The following table summarises the mode of embryo development in the Cyperaceae (Didricshen, 1894, 1897; Guignard, 1961; Gupta, 1962; Guttenberg and Semlow, 1957; Heilborn, 1918; Juguet, 1966; Juguet and Lebégue, 1966; Khanna, 1965; Murty and Kumar, 1967; Padhye, 1960; Radhey Shiam, 1963; Shah, 1959, 1965; Shah and Neelakandan, 1971; Veken, 1965) :

---

* Some members of Epacridaceae show the nuclear migration and the death of 3 meiotic products, but their development is different (Smith-White, 1959).

TABLE I

CELL GENERATIONS IN EMBRYO-GENESIS


I
II
III
IV
Final fate
l—cotyledon+Upper lip of the cotylar sheath
ca — q — octant
l' shoot apex + hypocotyl
Zygote
iec
m
ico
lower lip of the cotylar sheath + radicle + major part of the root cap
cb
n — n – part of the root cap
ci
o
n'
suspensor
p

The zygote divides transversely resulting in cells *ca* and *cb*. The cell *ca* gives rise to the quadrant contributing to the cotyledon and the shoot apex, *cb* on the other hand undergoes a transverse division and forms the radicle and the suspensor. It may be said further that the cells of the quadrant cut off dermatogen initials very early, just as in the family Juncaceae, and the embryogeny may be classified, therefore, under the Juncus variation of the Onagrad type (Johansen, 1950). An isolated instance of Asterad type has been claimed by Khanna (1965) for *Scirpus mucrinatus* but her figures are not convincing and the development of the mature embryo could not be followed by her to trace the destiny of each cell. The initiation of the shoot apex is marked by an indentation between tiers *l* and *l'*. The upper lip of the cotylar sheath forms from the tier *l*, while the lower lip is differentiated from the tier *m*. Both the lips together encircle the shoot apex which originates from the tier *l'*. Again such a situation has a parallel only in the Juncaceae (Souèges, 1923, 1933).

As early as 1892, Wilczek sketched an embryo of *Scirpus*. His illustrations indicate that the organ considered to be radicle is in fact a part of the cotyledonary sheath and that the so-called first lateral root is in reality the true radicle (Shah, 1963). This erroneous interpretation seems to be due to an abnormally well-developed cotyledonary sheath. Schneider (1932) noted three different types of embryos in Cyperaceae, Carex, Cyperus and Scirpus types. It is obvious that the embryo of *Scirpus* is identical with that of *Cyperus* and therefore the Scirpus type is not a valid category. But instead of Scirpus type, we must erect another category—the Bulbostylis type where both the radicle and plumule point towards the micropyle, while in *Schoenus* (Veken, 1965) and *Scleria* the cotyledon is terminal and the plumule and the radicle are lateral.

The disposition of the different organs in the mature embryo is variable in the Cyperaceae. There is a gradual deflection in the position of the radicle. In *Carex* the plumule is lateral, whereas the radicle and the cotyledon are terminal and lie in the same axis; in *Bulbostylis*, both the plumule and the radicle point towards the micropyle and cotyledon is terminal as in *Carex*; in *Cyperus* the plumule faces the micropyle and it is the radicle which is lateral and shifted to one side (Shah, 1963); in *Schoenus* both plumule and radicle are lateral and cotyledon is terminal (Veken 1965; Shah and Neelakandan, 1971).

To conclude, therefore, the Cyperaceae reveals the following distinct types of embryos (Table II):

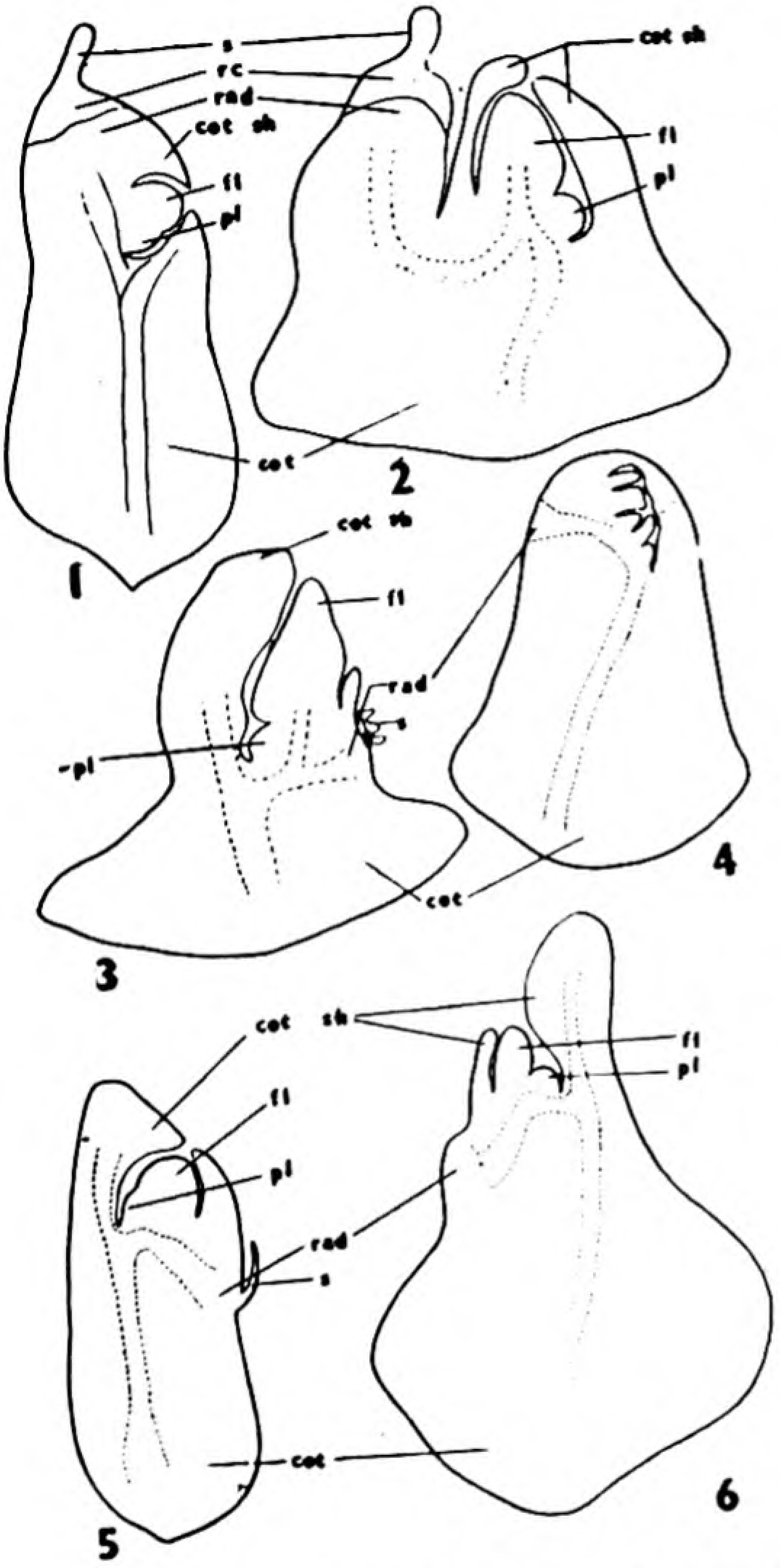
To Veken (1965), it appears that a natural genus contains only one embryo type, but a given type may be shared by several genera though varying in secondary characters throughout the genus. The embryos of 132 species of *Cyperus* (Veken, 1965) were all of the same Cyperus type. This uniformity conforms to the wide conception of the genus *Cyperus* and supports the inclusion of the genera *Pycreus, Juncellus* and *Mariscus* under the genus *Cyperus*. In this regards, Britton's (1907) statement is quite informative, "I have accepted the view of the previous authors in taking *Cyperus* in its broad sense rather than recognising the genera *Pycreus*, *Mariscus*, *Torulinium*, as I believe the characters depended on by Clarke (1884) for the maintenance of these genera are too vague for satisfactory use." The embryography of *Kyllinga*

TABLE II

EMBRYOGRAPHY

Type	Plumule	Radicle	Cotyledon	Examples
1. *Carex* type:	Lateral	Basal	Terminal	*Carex wallichiana* (Shah, 1965).
2. *Cyperus* type:	Basal	Lateral	Terminal	*Cyperus, Kyllinga, Eleocharis* (Shah, 1960, 1962c, 1964); *Fimbristylis* (Murty and Kumar, 1967); *Scirpus* (Khanna, 1965); *Ascolepis, Lipocarpha, Hypolytrum* (Juguet, 1970).
3. *Bulbostylis type*:	Basal	Basal	Terminal	*Bulbostylis barbata* (Shah, 1965).
Schoenus type:	Lateral	Lateral	Terminal	*Schoenus* (Veken, 1965), *Scleria hebecarpa* (Shah and Neelakandan, 1971).

supports its merger with the genus *Cyperus* (Shah 1960; Khanna, 1965).



FIGS. 1-6. Mature embryos of the Cyperaceae in L.s. Fig. 1. *Carex wallichiana*. Fig. 2. *Bulbostylis barbata*. Fig. 3. *Eleocharis palustris*. Fig. 4. *Scleria hebecarpa*. Fig. 5. *Cyperus articulatus*. Fig. 6. *Eleocharis plantaginea*.

On the contrary, the genus *Scirpus* showed six embryo types (Veken, 1965). Sections containing more than one embryo type appeared to be heterogeneous as to flower morphology too and were spilt. Thus embryography supports splitting of *Scirpus* in at least six genera. The taxa *Bulbostylis* (Shah, 1965), *Abildgaardia* and *Actinoschoenus* (Veken, 1965) should not be included in *Fimbristylis* as they have an embryo type quite different from that of the bulk of the *Fimbristylis* species.

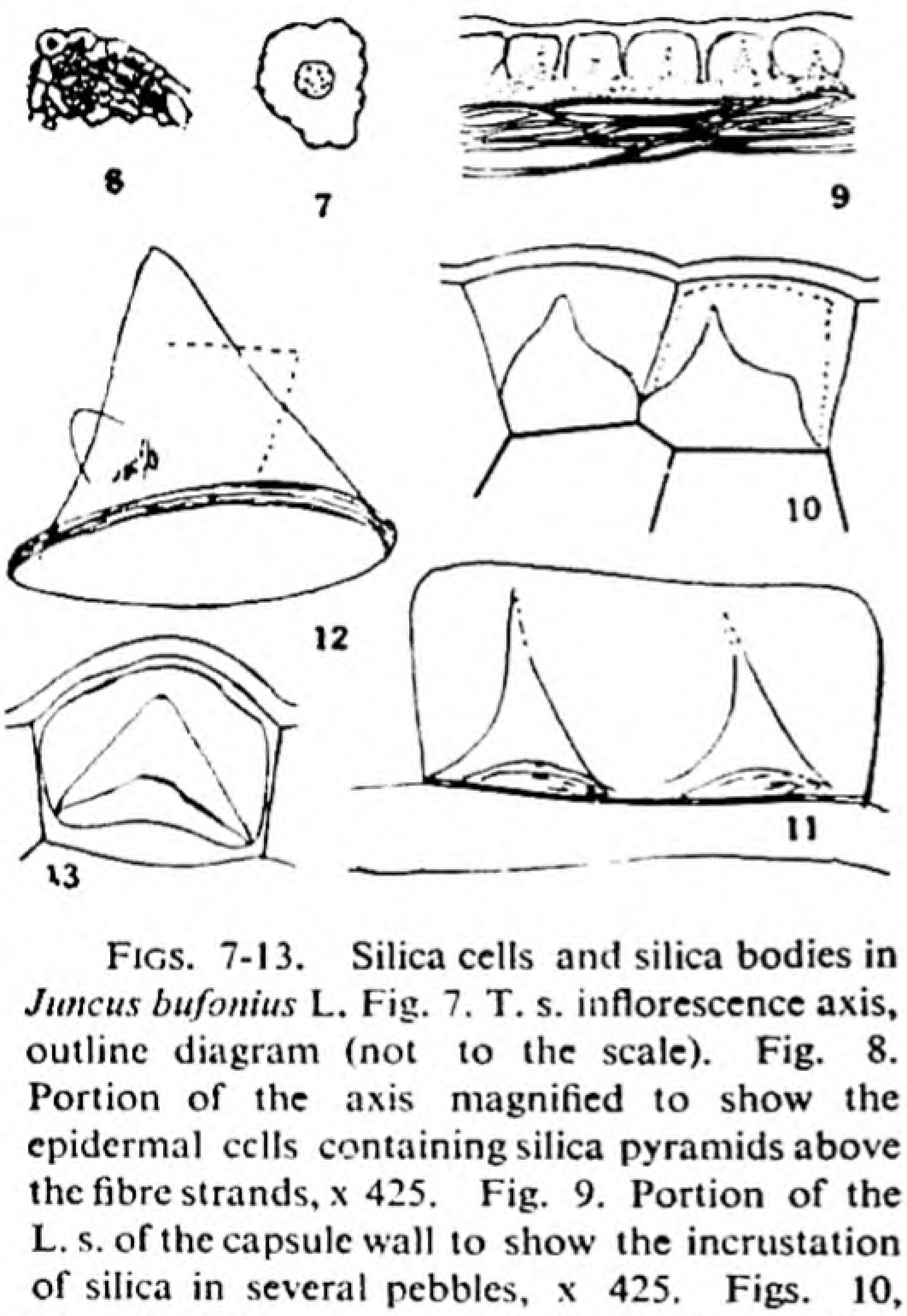
SEED COAT AND PERICARP

The ovule is anatropous, bitegminal and crassi-nucellar (Marek, 1958; Shah, 1968). The inner integument becomes 3- or 4-layered at the micropylar end. Tannin accumulates in nucellar cells at the chalazal end, where a hypostase is formed. From this centre, tannin spreads into the outer layer of the inner integument and the inner layer of the outer integument. The testa and tegmen are coalescent and form a thin skin. They also exhibit cutin-like thickenings and develop undulating folds.

The ovary wall comprises the outer epidermis 5 or 6 middle layers and an inner epidermis. The middle layers develop into fibres. In layers proximal to the outer epidermis of the ovary, the fibres are arranged longitudinally, whereas in the layers distal to the outer epidermis of the ovary, they are transversely arranged. However, not all the middle layers become fibrous, for some of them degenerate during earlier stages of development. Studies on macerated pericarp have clearly revealed the branched nature of some of the fibres. The cells of the outer epidermis show silica pyramids and their granulated peg-like thickenings project into the cell lumen (Wilczek, 1892). The testa is free from the pericarp.

The dry material of one plant each of *Juncus bufonius* (Shah, 1963) contained

1.08 per cent by weight of silicon dioxide. *Juncus interior* Wieg., which grows in Kansas also contains silica but only about half as much (Lanning, 1971). Thus the quantitative determinations reveal silica in Juncaceae. Silica crystals, quite common in the Cyperaceae (Metcalfe, 1963), are also observed in Juncaceae (Figs. 7-13). Thus the findings help to bridge the two families firmly and the main anatomical difference between the two families vanishes (Metcalfe, 1969).



FIGS. 7-13. Silica cells and silica bodies in *Juncus bufonius* L. Fig. 7. T. s. inflorescence axis, outline diagram (not to the scale). Fig. 8. Portion of the axis magnified to show the epidermal cells containing silica pyramids above the fibre strands, x 425. Fig. 9. Portion of the L. s. of the capsule wall to show the incrustation of silica in several pebbles, x 425. Figs. 10, 11, 13. T. s. stem or leaf showing epidermis containing pointed silica deposition, x 925. Fig. 12. A single isolated silica pyramid with accessory projections, x 1500.

MESOCOTYL

During germination, the region in between the cotylar sheath (Fig. 14) and the haustorial part of the cotyledon elongates considerably and get interpolated with the plumule-radicle axis (Shah, 1962d). This axis was termed a "mesocotyl" by Celakovsky (1897, cited from Goebel, 1905). Subsequently the term has been interpreted in a variety of ways (Roth, 1955) which has created confusion.

My observations (Shah, 1962d) on it may be summarised as follows:

(i) The mesocotyl separates the cotyledonary sheath from the haustorial portion of the cotyledon.

(ii) It shows a uniform cortical tissue through which passes a median bundle of the haustorium.

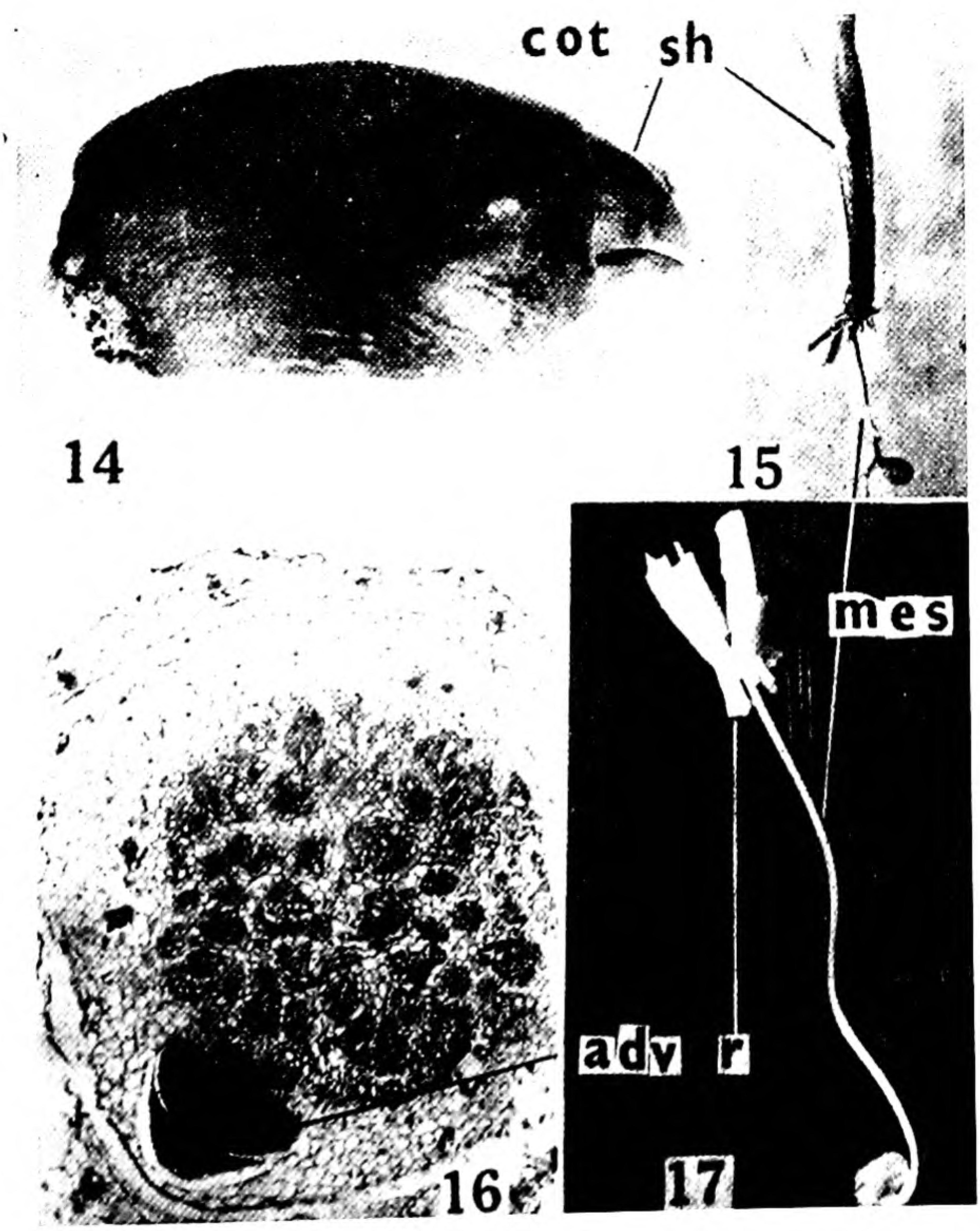
(iii) The vascular bundle runs from the haustorium to the cotyledonary sheath. It is conjoint and collateral.

(iv) There is a central stele known as the mesocotylar stele. Adventitious roots arise at the tip of the mesocotyl (Fig. 16).

(v) The stele is tetrarch and exarch. There is no pith.

However, Guttenberg and Semlow (1957) reported the presence of pith in the mesocotylar stele and Arber (1925) observed a bicollateral bundle in the mesocotyl of *Carex*.

I am tempted to concur with Roth (1955) that the mesocotyl is neither a hypocotyl nor an epicotyl, neither a node nor an internode. It is an axis with characteristics of its own. It is situated between the cotyledonary sheath and the haustorial portion of the cotyledon (Figs. 15, 17). Any interpretation of the mesocotyl on the aforesaid terms leads to the conclusion that the cotyledonary sheath is a leaf which is absurd, because a leaf cannot possibly arise from another leaf (the cotyledon). The other point is that the mesocotyl has a stele like that of a root. Hence it is not a stem.



FIGS. 14-17. Mesocotyl. Fig. 14. Mature seed of *Remirea maritima* showing the emergence of the coleoptile first from the seed coat, x 20. Fig. 15. Seedling of *Cyperus rotundus*. Note the axis of the mesocotyl separating the seed and the young plant, x 8. Fig. 16. T. s. passing through the junction of the mesocotyl and the transition region showing the origin of adventitious roots, x 480. Fig. 17. Adventitious roots developing from the tip of the mesocotyl, x 20.

adv. r, adventitious root; *cot. sh.* cotyledonary sheath; *mes*, mesocotyl

CONCLUDING REMARKS

Alliance of the two families Cyperaceae and Gramineae in a single order is open to question (Shah, 1967). The superficial grasslike habit in Cyperaceae is of no phylogenetic significance and has appeared also in other unrelated families. The leaf sheaths are closed so that each one completely encircles the stem in the sedges. In the grasses, the sheaths are open on one side. In the sedges the two or three coalescent carpels have corresponding number of stigmas and none lack stigmas as in grasses. Further the spikelets of the Cyperaceae are not at all homologous with those of the grasses, since in the former they vary widely in organisation within the family whereas in the latter they are resonably constant. The florets of the Gramineae (excluding bracts) are borne terminally, whereas in the Cyperaceae they are always axillary in position (Shah, 1962b).

The clinching evidence for the close relationship between Cyperaceae and Juncaceae comes from their embryology and cytology. The Juncaceae produce [illegible] [illegible] [illegible] the Cyperaceae, [illegible] [illegible] [illegible], embryological [illegible] [illegible] [illegible] that the sedge [illegible] [illegible] [illegible] containing the usual vegetative and generative cells plus three non-functioning nuclei of the other three microspores of the tetrad. Simultaneous cytokinesis, tenuinucellar ovules with obturator, embryogeny of the Juncus variation of the Onagrad type with periclinal divisions in the quadrant bind the two families firmly (Maheshwari, 1964). Further, members of both the Juncaceae and the Cyperaceae have chromosomes with diffuse centromeres and post-reductional meiosis (Battaglia and Boyes, 1955) a rather rare condition among angiosperms, resulting in intense aneuploidy. However, migration of the 3 nuclei to die due to plasmolysis, only one functional microspore inside a common exine, re-arrangement of the 3 nuclei into discrete protoplasts during expansion of the pollen mother cells, cytokinesis simultaneous but extremely delayed, abortive divisions of the 3 nuclei, 3-celled monocolpate pseudomonad, embryo four types—Bulbostylis, Carex, Cyperus and Schoenus, a mesocotylar procambial strand in the embryo, and pericarp and testa separate (Marek, 1958) are the additional entities to bind the 3 sub-families firmly into a well-knit unit. Hence Lawrence (1951) and Meeuse (1965) contention to split the family sounds untenable.

REFERENCES

[illegible] Morpholo- [illegible]

[illegible] 55. Post- [illegible] and caus [illegible]

BRIT[illegible] of Jamaica. *Bull. Dept* [illegible]

CARNIEL, K. [illegible] lungsgeschiche des [illegible] Gramineen und Cy[illegible] *bot. Z.* **109**: 21-95.

——. 1962b. Die Entwick[illegible] petums der Cyperaceen [illegible] Kernen mit diffus—centrom[illegible] chromosomen. *Öst. bot. Z.* **109**: 168-1[illegible].

CLARKE, C. B. 1884. On the Indian species of *Cyperus* with remarks of some others that especially illustrate the sub-divisions of the genus. *J. Linn. Soc. Bot.* **21**: 1-102.

DIDRICSHEN, A. 1894, 18[illegible]. On Cyperaceernes Kim. *Bot. Tidsskr.* **19**: [illegible]; **21**: 1-[illegible]0.

DNYANSAGAR, V. R., AND D. K., TIWARI 1956. Sporogenesis and gametophytes of *Fimbristylis quinquangularis* Kunth. *Curr. Sci.* **25**: 16.

——. 1956. Sporogensis and gametophytes of *Fimbristylis quinquangularis* Kunth. *Bull. bot. Soc. Univ. Saugar.* **8**: 3-6.

GOEBEL, K. 1905. *Organography of Plants.* Part II. Oxford.

GREZICKA, W. 1964. Megasporogenesis, ovule and embryo sac development in *Carex aristata* R. Br. var. *cujavica*. *Acta. Soc. Bot. Pol.* **33:** 307-322.

GUIGNARD, J. L. 1961. Developpement de l'embryon chez le *Cyperus vegetus* Wild. *C. r. Acad. Sci., Paris.* **252:** 2125-2127; *Ann. Sc. nat. Bot.* **2:** 560:-562.

GUPTA, M. N. 1959. Structure of the flowers and the development of gametophytes in *Kyllinga triceps* Rottb. *Proc. 45th Indian Sci. Congr. Madras,* 283-284.

——. 1962. Morphological studies in Cyperaceae. I. Development of the ovule and the gametophytes in *Fimbristylis dichotoma* Vahl. *Agra. Univ. J. Res.* **(*Sci.*) 11:** 59-67.

GUTTENBERG, H. V., AND A. SEMLOW. 1957. Die Entwicklung des Embryos und der Keimpflanze von Cyperaceen. *Bot. Stud. Jena.* **7:** 127-141.

HEILBORN, O. 1918. Zur Embryologie und Zytologie einiger *Carex*-arten. *Svensk bot. Tidskr.* **12:** 212-224

HOLTTUM, R. E. 1948. The spikelet in Cyperaceae. *Bot. Rev.* **14:** 525-451.

HUTCHINSON, J. 1969. *Evolution and Phylogeny of Flowering Plants.* Academic Press, London.

JOHANSEN, D. A. 1950. *Plant Embryology.* Waltham. Mass. U. S. A.

JUGUET, MARC. 1966. Embryogenie des Cyperacees. Développement de l'embryon chez le *Scirpus martimus* L. *C. r. Acad. Sci. Paris.* **263:** 1710-1713.

——. 1966. Embryogènie des Cypèracees. Development de l'embryon chez le *Carex arenaria* L. *C. r. Acad. Sci., Paris* **262:** 1844-1947.

——. 1970. Development de l'embryon chez quelques Cypéracees Africaines. *Adansonia* **10:** 271-288.

——, AND A. LEBEGUE, 1966. Embryogenie des Cyperacees. Development de l'embryon chez le *Carex arenaria* L. *C. r. Acad. Sci., Paris.* **262:** 1844-1847.

KERN, J. H. 1962. New look at some Cyperaceae mainly from the tropical stand point. *Advance Sci.* **18:** 141-148.

KHANNA, P. 1963. Male gametophyte and obturator in *Cyperus rotundus* L., and *C. triceps* Endl. (=*Kyllinga triceps Rottb.*). *Curr. Sci.* **32:** 133-134.

——. 1965. A contribution to the embryology of *Cyperus rotundus* L., *Scirpus mucrinatus* L., and *Kyllinga melanospora* Nees. *Can. J. Bot.* **43:** 1539-1547.

KUBITZKI, K. 1966. Untersuchungen über den Blütenbau von *Oreobolus* R. Br. *Bot. Jb.* **85:** 80-87.

LANNING, F. C. 1971. Personal communication dated July 21, 1971.

LAWRENCE, G. H. M. 1951. *Taxonomy of Vascular Plants.* New York.

LEWIS, K. R., AND B. JOHN 1961. Hybridisation in a wild population of *Eleocharis palustris. Chromosoma* **12:** 433-448.

MAHESHWARI, P. 1964. Embryology in relation to taxonomy. In *Vistas in Botany* Vol. 4 W. B., Turril (ed.). London. 55-97.

MAREK, S. 1958. A study of the anatomy of fruits of European genera in the subfamilies Scirpoideae Pax, Rhynchosporoideae, Aschers,. et Graebner and some genera of Caricoideae Pax. *Monographiae bot.* **6:** 151-177.

MEEUSE, A. D. J. 1965. Angisoperms—past and present. Institute for the Advancement of Sci. Cult., New Delhi.

METCALFE, C. R. 1963. Comparative anatomy as a modern botanical discipline, with special reference to recent advances in the systematic anatomy of monocotyledons. *Adv. Bot. Res.,* **1:** 101-147.

——. 1969. Anatomy as an aid to classifying the Cyperaceae. *Am. J. bot.* **56:** 782-790.

MURTY, Y. S., AND V. KUMAR. 1967. Development of the female gametophyte and embryo in *Fimbristylis diphylla* Vahl. *Proc.* 54th *Indian Sci. Congr., Hyderabad.* 323.

PADHYE, M. D. 1960. A contribution to the life history of *Kyllinga triceps* Rottb. *Bull. bot. Soc. Coll. Sci., Nagpur* **1:** 1-15.

——. 1966. The pollen grains of *Kyllinga* Rottb. *Paly. Bull.* **2, 3:** 101-103.

——, AND MOHARIAR, S. K. 1958. Studies in the embryology of *Cyperus tegetum* Roxb. *Proc. Indian Acad. Sci. B.* **48:** 89-96.

PIECH, K. 1924. Zur Entwicklung der Pollenkörner bei *Scirpus lacustris* L. *Bull. Acad. bot. Sci. Lettres, Cl. Sci. Math. Nat.* **1924:** 113-123.

——. 1928. Zytologische studien an der Gattung *Scirpus. Bull. Acad. bot. Sci. Lettres, Cl. Sci. Math. Nat.* **1928:** 1-43.

RADHEY SHIAM. 1963. Monographic studies of *Cyperus esculentus. Agra Univ. J. Res.* **12:** 181-184.

ROTH, I. 1955. Zur morphologischen Deutung de Grassembryos und verwandter Embryotypen. *Flora., Jena* **142:** 564-600.

SCHNEIDER, M. 1932. Untersuchungen Über die Embryobilung und Entwicklung der Cyperaceen usw. *Beih. bot. Zbl.* **49:** 649-674.

SHAH, C. K. 1959. *Morphological and embryological studies in the families Cyperaceae and Juncaceae.* Ph. D. Thesis, Univ. Delhi.

——. 1960. The embryology of *Kyllinga triceps* Rottb. *Vidya J. Gujarat Univ.* **3:** 61-74.

——. 1962a. Pollen development in some members of the Cyperaceae. In *Plant Embryology.* A Symposium, 1960. CSIR, New Delhi 81-83.

——. 1962b. Floral morphology of the Cyperaceae. I. *Le Naturaliste Canadien* **89:** 330-341.

——. 1962c. The life history of *Cyperus niveus* Retz. *Vidya J. Gujarat Univ.* **5:** 148-158.

——. 1962d. Studies in germination I. *Carex wallichiana* Presc. (*Carex fedia* Nees ex Wight). *J. Indian bot. Soc.* **41:** 551-556.

——. 1963 The life history of *Juncus bufonius* L. *J. Indian bot. Soc.* **42:** 238-251.

——. 1964. The embryo development in *Eleocharis palustris* L. *Le Naturaliste Canadien* **91:** 41-49.

——. 1965. Embryogeny in some Cyperaceae. *Phytomorphology* **15:** 1-9.

——. 1967a. Pollen development in some Cyperaceae. II. Phase of contraction and abnormal pollens. *Vidya J. Gujarat Univ.* **10** 236-241.

——. 1967b. A taxonomical evaluation of the families Cyperaceae and Juncaceae. *Sym. Newer trends in Taxonomy. Nat. Inst. Sci. India, New Delhi* **34:** 248-256.

——. 1968. Development of pericarp and seed coat in the Cyperaceae. *Le Naturaliste Canadian* **95:** 39-48.

——, and N. Neelakandan. 1971. Embryogeny in some Cyperaceae II. *Beitr. Biol. Pfl.* **47:** 215-227.

Smith-White, S. 1959. Pollen development patterns in the Epacridaceae. A problem in cytoplasm-nucleus interaction. *Proc. Linn. Soc. N. S. W.* **84:** 8-35.

Soueges, R. 1923. Embryogénie des Jonacées. Dévelopment de l'embryon chez le *Luzula forsteri* DC. *C. r. Acad. Sci., Paris.* **177:** 705-708.

——. 1933. Recherches sur l'embryogénie des Joncacées. *Bull. Soc. bot., Fr.* **80:** 51-59.

Strandhede, S. O. 1965. Chromosome studies in *Eleocharis,* sub. sr. *palustris II.* Pollen mitosis with special reference to some strains with 15 chromosomes and formation of secondary unreduced pollen grains. *Hereditas* **53:** 374-388.

Süessenguth, K. 1954. Systematik der Spermatophyta. *Fortschr. Bot. Berl.* **15:** 24.

Takhtajan, A. 1959. *Die Evolution der Angiospermen.* Jena.

——. 1969. *Flowering plants—Origin and Dispersal.* Translated by C. Jeffrey. Oliver and Boyd, *Edinburg.*

Tanaka N. 1941. Chromosome studies in Cyperaceae. XII. Pollen development in five genera with special reference to *Rhynchospora. Bot. Mag., Tokyo* **55:** 55-65.

——. 1950. Gametogenesis and fertilization in the genus *Carex. Coord. Comm. Res. Genetics* **1:** 133-137.

Thorne, R. F. 1963. Some problems and guiding principles of Angiosperm phylogeny. *Am. Nat.* **47:** 287-305.

Van Der Veken, P. 1965. Contribution A. L'embryographic systematique des. Cyperaceae-Cyperoideae. *Bull. Jard. bot. Etat. Brux.* **25:** 143-147.

Wilczek, E. 1892. Beiträge zur Kenntniss des Baues der Frucht und des Samens der Cyperaceen. (Beih) *Bot. Zbl.* **51:** 129-138; 193-201; 225-233; 257-265.

Adv. Pl. Morph. 1972: 113-125

THE EARLY DEVELOPMENT OF THE GRASS EMBRYO AND ENDOSPERM[1]

BY KNUT NORSTOG

Northern Illinois University, Cekalb, Illinois. U.S.A.

ABSTRACT

Endosperm (rye-grass. barley) and embryo of barley differ in several developmental aspects. Egg and central cell ultrastructure (barley) differs as does that of endosperm and embryo, and divergent development appears to be established prior to double fertilization. Both embryo and endosperm may be cultured on similar media but respond differently, the former differentiating on auxin-less media and callusing on auxin-containing media, the latter forms only callus and is independent of exogenous auxin.

One of the most interesting and yet perplexing phenomena in botany is double fertilization in angiosperms. Two cells, the central cell and the egg (Fig. 1, 2), both of which are derived from the same parent cell, are fertilized by sperm cells from the same pollen tube but undergo completely different developmental pathways. Brink and Cooper (1947) state the paradox succinctly in their review, to which the reader is referred for an extensive treatment of endosperm development: "The embryo embodies the line of descent and is the principal component of the seed. Endosperm, nucellus and integument are ancillary to the embryo in maintaining the succession." Not only do these roles provide greatly different destinies, but also diverging developmental pathways. For instance, in barley, the principal subject of this paper, as well as in many other angiosperms (Maheshwari, 1950) the initial divisions of the zygote involve cytokinesis, while those of the endosperm are free nuclear divisions. Subsequently, when the endosperm becomes cellular, a different mechanism of wall formation occurs. Our electron micrographs suggest that this occurs by aggregation of vesicles secreted by the rough endoplasmic reticulum, whereas wall formation in dividing embryo cells occurs as a consequence of phragmoplast development and involves the Golgi complex as reported in maize root tips by Whaley and Mollenhauer (1963).

The preoccupation in some laboratories, such as our own, with embryo and endosperm development has led to the employment of embryo and tissue culture techniques, with the assumption that it may be possible to find explanations for developmental differences by reducing the number of variables one must contend with. Explanting embryos and endosperm to nutrient media has, in fact, elucidated some aspects of development, but unfortunately has not solved a number of fundamental questions, chief of which are the biochemical and molecular bases for developmental divergence. The studies furnishing the data presented in this paper have

---

[1] Much of this study was supported by successive grants (currently GB-6759) from the National Science Foundation, Washington, D.C.

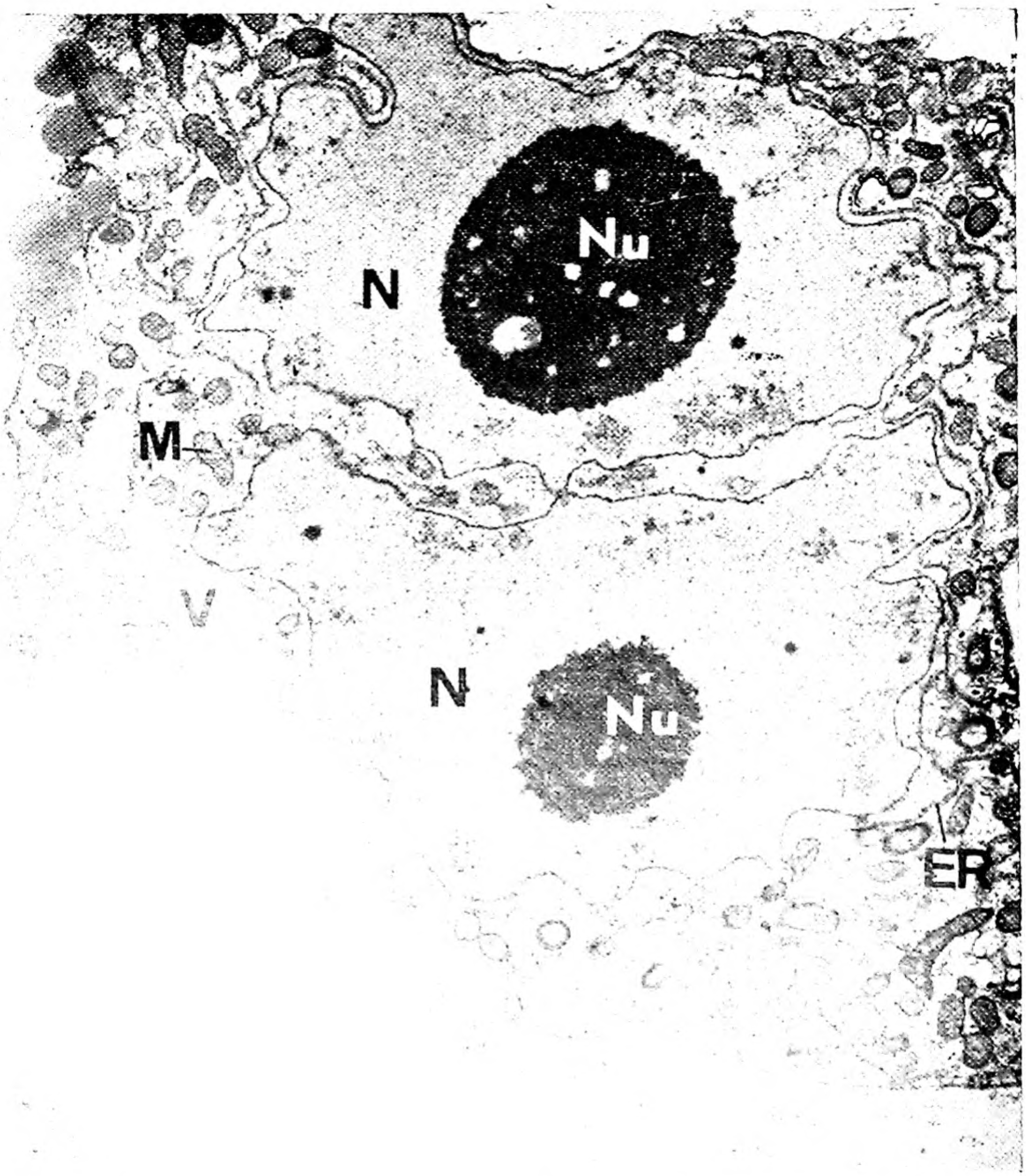


FIG. 1. [illegible] of ovule including the two polar nuclei, x6400. ER, [illegible] M, mitochondrion; N, nucleoplasm; Nu, nucleolus; P, plastid [illegible]

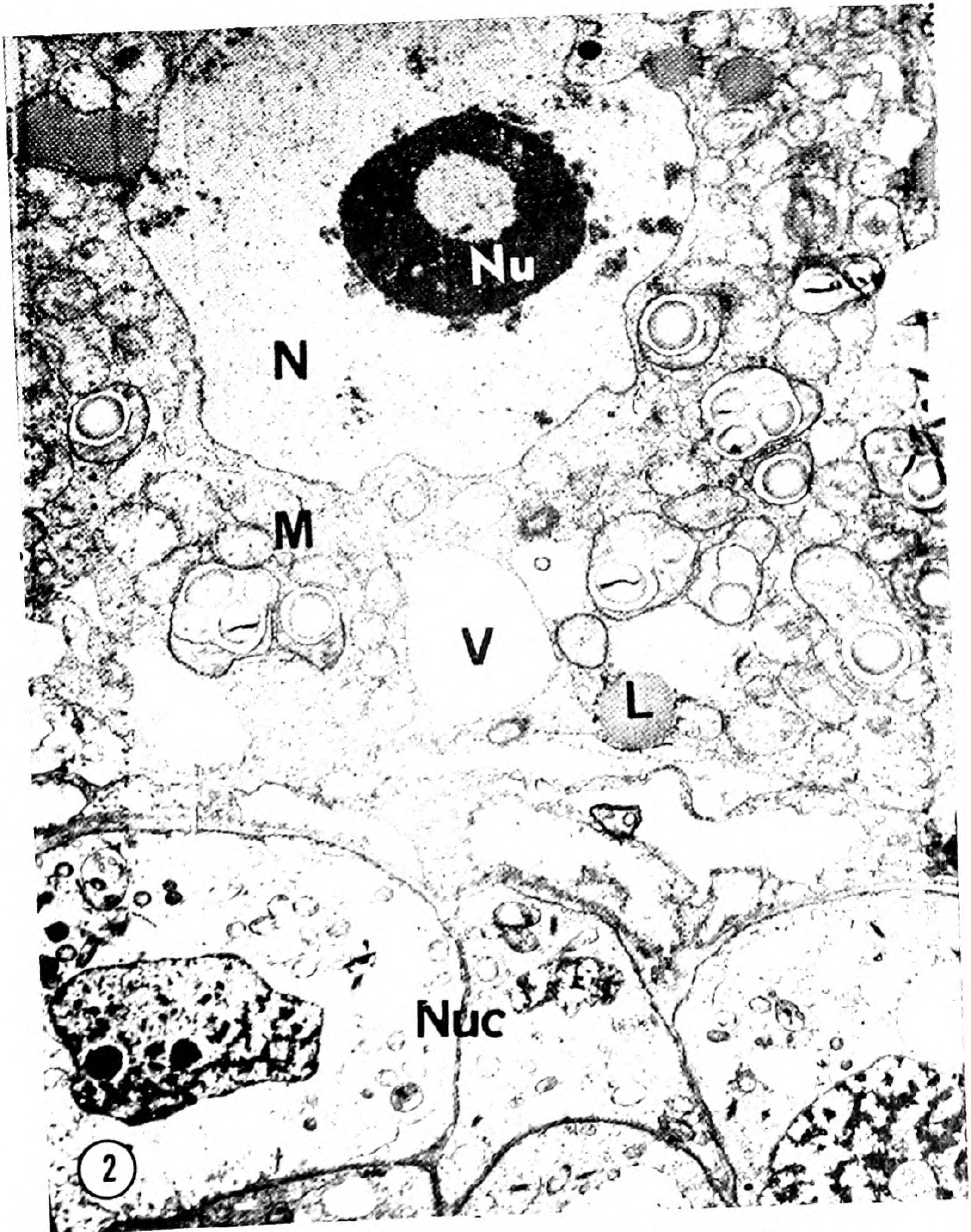


FIG. 2. A portion of the egg cytoplasm of barley including the nucleus, x6400. L, lipid drop; M, mitochondria; N, nucleoplasm; Nu, nucleolus; V, vacuole.

been in progress for a number of years and deal specifically with the endosperm and embryo of barley, *Hordeum vulgare,* and the endosperm of rye grass, *Lolium perenne.*

MATERIALS AND METHODS

Embryos and endosperm of barley, *Hordeum vulgare* L. cv. "Moore" have been excised and cultured on a variety of media including both defined (Norstog, 1967; Norstog and Smith, 1963) and undefined (i.e. coconut milk: Norstog, 1956a, 1961). The cultures herein described were made exclusively with a defined medium (Medium J in Norstog, 1967) which consists of a modified White's mineral solution (White, 1943), vitamins including inositol, ammonium malate, and an amino acid mixture. Regarding the latter, glutamine (400 mg/1) appears to be essential; several other amino acids are also included but are perhaps not essential. The modification of the mineral solution consisted chiefly of an increase in the potassium salts. Generally the medium was used without added hormones, but in some cultures 2,4-D, IAA, kinetin, and gibberellic acid have [illegible] or in combination. This [illegible] been used to culture [illegible] in the presently reported [illegible] ments, *Euphorbia* [illegible] communication [illegible] sustaining [illegible] personal [illegible]

R[illegible] cultured [illegible] medium (Norst[illegible] 1969) and on a [illegible] on the barley embryo [illegible] described above, and differs from it in having a reduced sucrose concentration (2% vs. 6.8%). Hormones, including IAA, kinetin and gibberellic acid singly or in combination, were added in some cultures. Cultures were also made on the high salt medium of Linsmaier and Skoog (1965). The endosperm was initially isolated in 1955 and has been maintained since that time by frequent subculturings. During this time there has been a gradual diminution of its capacity for rapid growth (see Norstog et al., 1969) so that at present a four- to five-fold increase in wet weight in 20 days is obtained — considerably less than in the original cultures (see Table 1). Cultures of rye-grass endosperm were also made on White's medium containing several amino acid mixtures as well as individual amino acids. Many of these cultures were done in 1959 and 1960 in an only partially successful attempt to develop a defined medium. They now appear significant in light of present successful cultures of the endosperm on defined media.

RESULTS

Ultrastructure—In figures 1 and 2 the ultrastructure of the barley egg may be compared with that of the polar nuclei and surrounding cytoplasm. Note that the endoplasmic reticulum is in evidence in the central cell cytoplasm and not in the egg. Lipid drops are present in both. The nucleoli are somewhat larger in the polar nuclei than in the egg, and lack a central, light-staining region. The mitochondria are large and irregular in the egg, smaller, more or less isodiametric and more numerous in the central cell. Central cell mitochondria also have a more densely staining matrix and more cristae than those of the egg, *suggesting that they may be more active in respiratory metabolism than those of the egg.* The egg cytoplasm is more vacuolate than that of the central cell. The plastids of the former, unlike the latter, contain starch grains. The presence of rough endoplasmic reticulum in the central cells suggests a more active enzyme biosynthesis than in the egg in which the endoplasmic reticulum is not observed.

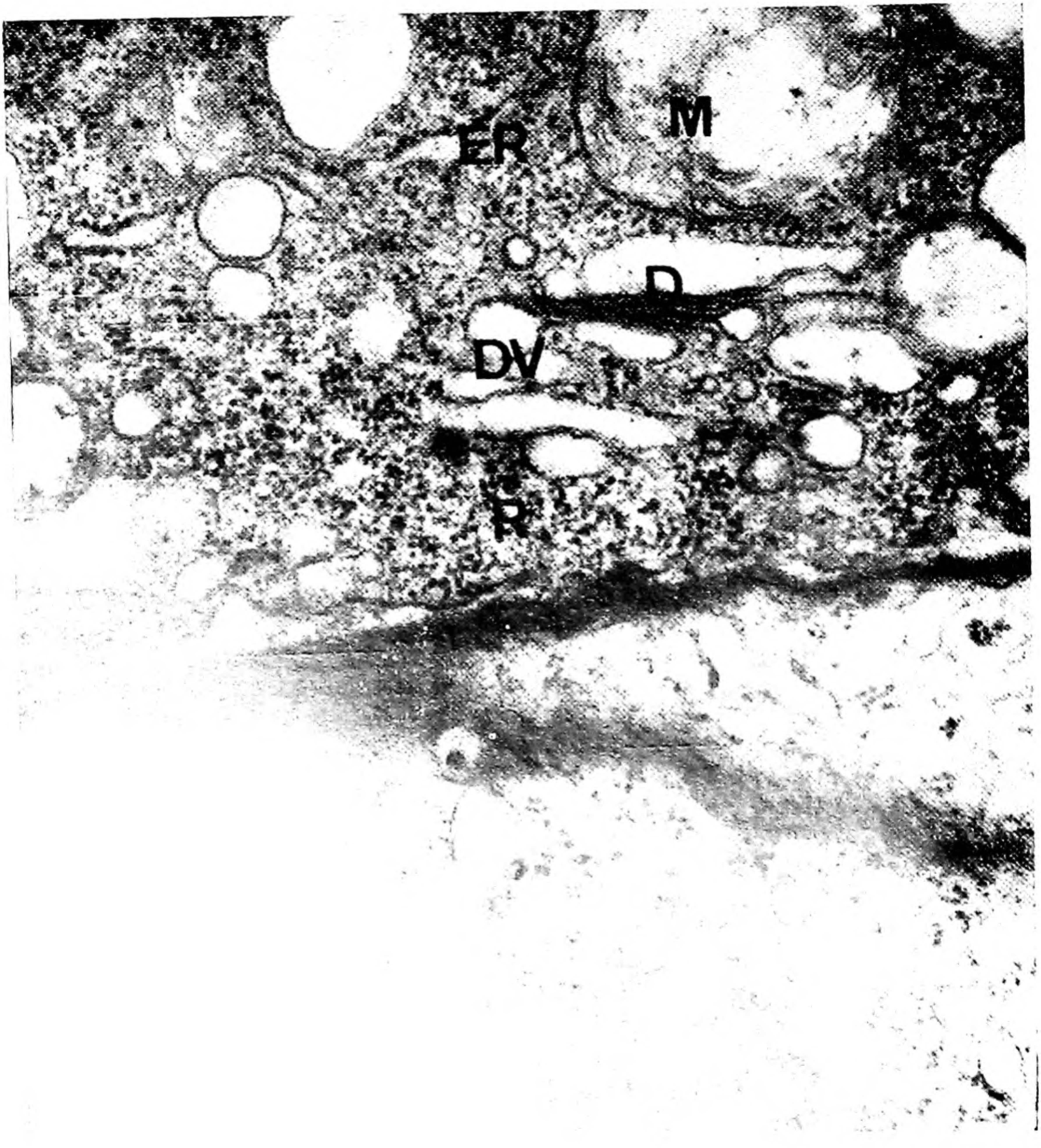
TABLE I

GROWTH OF RYE-GRASS ENDOSPERM ON VARIOUS MEDIA

Medium[1]	Inoculum wt.	Increment wt.	Increase	Comparative yield[2]
		20-day cultures (1959-60)		
BY[3]	1.978 grams	18.726	947%.	100%
B+AA[4]	3.262	16.865	517	55
B+AA[5]	1.512	5.714	378	40
B+Asp[6]	1.445	1.607	111	12
		20-day cultures (1970)		
B[3]	2.638	0.001	0	0
BY	2.231	7.129	320	100
BY−IAA	2.200	7.872	358	112
J-1[7]	3.132	14.766	471	147
LM[8]	3.702	1.761	48	15
		20-day cultures (1954)[9]		
BY	3.273	51.642	1578	100
B+Coconut milk	3.686	44.980	1220	77
		25-day cultures (1954-60)		
BY	2.021	43.383	2147	100
B+DNA	1.750	1.985	113	5
BY−IAA	3.917	56.377	1326	62
B+Asp[10]	2.016	10.874	539	25

1, All media contained 1 mg/l IAA unless specified; 2, Based on percent of increment with BY medium each group; 3, B = White's medium (White, 1943), BY-with 0.5% yeast extract and IAA 1 mg/l; 4, 30 mg/l each of 20 amino acids; 5, Amino acid mixture based on Chibnall's determination of those in orchard grass leaf, glutamic acid 100 mg/l all others in proportion; 6, Asparagine 30 mg/l; 7, Medium J in Norstog, 1967, except 2% sucrose, contains no IAA; 8, Linsmaier-Skoog (1965) medium, no auxin; 9, Reported in Norstog, 1956b. 10, Asparagine 200 mg/l.

Ultrastructural differences between endosperm (free nuclear stage) and embryonic cells (16—cell embryo) are also found (Fig. 3). The endosperm cytoplasm is literally packed with ribosomes. Rough endoplasmic reticulum is also more abundant than in embryo cells. The Golgi apparatus in the endosperm consists of dictyosomes with large secretion vesicles. The embryo cytoplasm has relatively fewer ribosomes and little endoplasmic reticulum, and dictyosomes when present, are not associated with large secretion vesicles. It appears, therefore, that both embryo and endosperm are engaged in synthesis of structural proteins upon free ribosomes



[illegible] endosperm and a portion of one cell of a 16-cell barley embryo [illegible] high concentration of ribosomes (polyribosomes) in endosperm [illegible]

D, dictyo[illegible]; R, polyribosomes; ER, endoplasmic reticulum.

(polyribosomes). However, the endosperm cytoplasm may be more active of the two since it possesses more ribosomes.

The significantly greater amount of rough endoplasmic reticulum and dictyosomes with large secretion vesicles in the central cell cytoplasm, as compared to the embryo, suggests greater activity of the former in bio-synthesis.

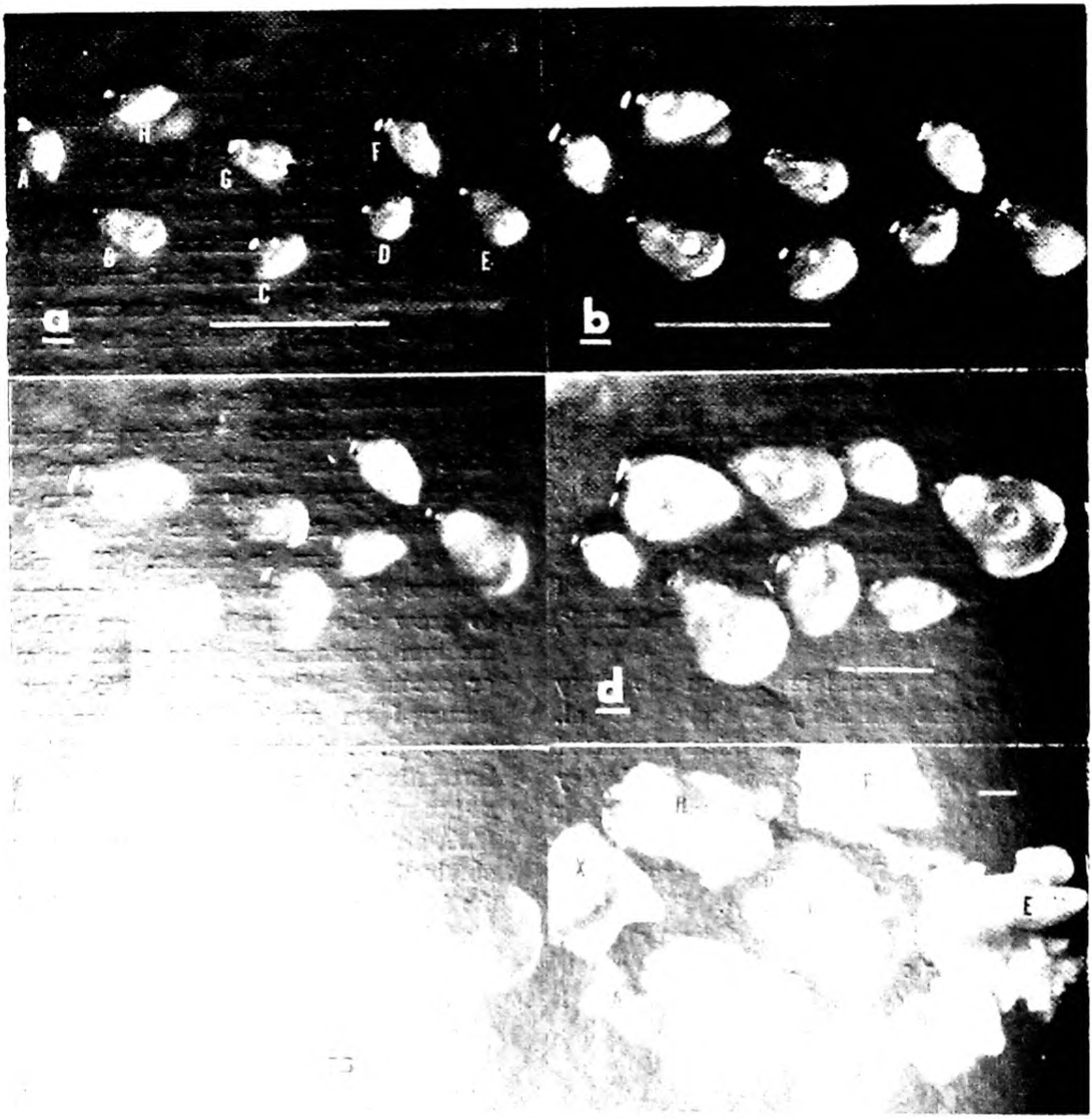
EMBRYO CULTURE

Initial attempts to culture barley embryos in early developmental stages (less than 0.5 mm long) either on synthetic media or those containing natural extracts were relatively unsuccessful, but did establish the necessity for maintaining a high osmolarity in culture media and a requirement for growth substances other than those present in simple defined media (sugar, mineral salts, and vitamins) (Ziebur and Brink, 1951).

A medium based in part on the assay of coconut milk by Tulecke et al. (1961) was developed which permitted essentially normal developmental barley embryos smaller than 0.5 mm (Norstog and Smith, 1963). Further modification (Norstog, 1967) permitted growth of embryos as small as 200μ. Figures 4a to 4f illustrate the growth of a set of 200μ proembryos over a period of days in which their day-to-day development was followed by time-lapse photography. Ongoing experiments with even smaller embryos (less than 100μ) indicate that they also may be grown on defined media but may require very low concentrations of IAA and kinetin (.01, .02 mg/l). At present, mechanical problems do not permit the culture of intact excised barley zygotes. Electron micrographs clearly indicate the attachment of the zygote wall to the nucellus. It does not appear to be possible to remove the zygote without damaging it in the process. Barley embryos, unlike some dicotyledon embryos (see Steward, et al., 1963; Blakely and Steward, 1964), cultured on media lacking hormones or having comparatively low hormonal levels have not produced a subculturable callus. However, embryos cultured in the liquid version of medium J together with high concentrations of 2, 4-D (10 mg/l) have produced subculturable callus masses (Fig. 5). Lower concentrations, of 2, 4-D (1 mg/l) result in the development of immense masses of roots upon cultured embryos (Fig. 6.)

Thus far, cultures of barley endosperm have not been productive; hence the reliance upon rye-grass endosperm for information regarding the behavior of grass endosperm in culture. The initial cultures of rye-grass endosperm appeared to grow about equally well on White's medium containing either yeast extract or coconut milk (Table 1). No growth occurred on media lacking these substances (i. e. a simple defined medium). Interestingly, the culture appeared to grow rather well on yeast extract media lacking IAA, although the yield was less than that obtained with IAA (1 mg/l). However, the inability of the tissue to maintain its growth on defined media excluded the possibility of making definitive statements regarding the influence of hormones and other naturally occurring substances on its growth and development. For this reason, attempts were made from time to time to develop a defined, synthetic medium capable of supporting vigorous growth and development of rye grass endosperm.

A series of cultures were attempted using various amino acids singly or in combination. In no case was growth activity comparable to that attained on


A
H
G
F
B
C
D
E
a
b
d
H
F
X
E

[illegible] medium J (see
[illegible] identified A-H,
are [illegible] A-D. White
line [illegible]

5

6

Figs. 5, 6. Fig. 5. Shake culture of barley callus derived from embryonic tissue. Cultured on medium J plus 10 mg/l 2, 4-D. x 1.5. Fig. 6. Shake culture of barley embryo callus on medium J plus 1 mg/l 2, 4-D. x 1.5.

the yeast extract medium (see Table 1). A mixture of 20 amino acids resulted in the production of a callus increment 55% that produced on the yeast extract medium. Cultures made with a medium containing an amino acid mixture based on Chibnall's (1939) assay, of the amino acids of orchard grass leaves were less effective, and it is interesting to note that this mixture of amino acids also failed to support the growth of young barley embryos (Norstog, 1961). Individual amino acids of this series also were ineffective compared to yeast extract.

A report by Strauss (1960) to the effect that asparagine was active in promoting the growth of maize endosperm suggested the use of higher levels of this amino acid than used in previous experiments. However, tests of the effect of asparagine on growth of rye-grass endosperm were not promising (Table 1). No other single amino acid was capable of supporting growth of the rye-grass tissue.

The attempts to develop a defined medium [illegible] abandoned at this point and the [illegible] maintained on the yeast [illegible] number of years. [illegible] were made [illegible] rye-grass [illegible] barley [illegible] singly (in [illegible] endosperm [illegible] that on [illegible]

The [illegible] those of [illegible] years the [illegible] city for rapid [illegible] is clear that [illegible] of supporting levels of growth equal to or better than that obtained in cultures on a yeast extract medium. Although no attempt has been made to determine which components of the defined medium are critical (that is, are absolutely required for growth), it is important to note that the tissue did not respond on White's mineral medium alone and cultures made with the high salt Linsmaier-Skoog medium were also unsuccessful. Since all data were recorded after the tissue had been subcultured twice on the test media, carry-over of yeast extract residues has been minimized, and it is believed that it can be stated quite accurately that the tissue is able to grow on an auxin-free medium (Table 1). The addition of IAA (1 mg/l) to the medium did not result in increased growth, and gibberellic acid and kinetin were also completely ineffective in promoting growth.

DISCUSSION

It is apparent that ultrastructural differences exist between the central cell and the egg of barley. The central cell cytoplasm contains more rough endoplasmic reticulum than the egg. Mitochondria are more numerous in the former, and their dense matrices and well developed cristae suggest greater respiratory activity than do those of the egg. It is likely that the smaller and more regular size of mitochondria is a consequence of rapid replication. The absence of starch granules from central cell plastids also may be a result of greater respiratory activity in that cell than in the egg. Differences in ultrastructure of central cell and egg have been reported in cotton (Jensen, 1965) and in maize (Diboll and Larson, 1966; Diboll, 1968). Perhaps the most significant interpretation of the distinctive ultrastructure of central cell and egg prior to their fertilization is that the divergent pathways of development exhibited by these cells and their progeny is not necessarily determined at fertilization. That is to say, double fertilization itself probably does not confer morphogenetic distinction upon these cells.

It has been shown that endosperm of a grass (rye-grass) is not unlike the embryo (barley) in its nutritional requirements. Although the role of components in culture media supporting growth of rye-grass endosperm has not been well-assessed, experience suggests that the organic milieu may play a larger part in growth than do modifications in the mineral solutions. Neither White's minerals alone (White, 1943) nor the high salt solution of Linsmaier and Skoog (1965) are capable of sustaining growth of rye grass endosperm. On the other hand, amino acids are known to sustain limited growth of grass endosperm. Of these, asparagine (Strauss, 1960) and glutamine are probably the most active. Their role in transaminations is well-known and may account for their action in supporting endosperm and embryo growth. The part played by other amino acids is less clear although some of them, for example cysteine, a component of our medium, counteract nitrate reductase inhibition by other amino acids (Filner, 1966). The endosperms of dicotyledonous plants also require amino acids for growth in vitro, as shown in their requirement for casein hydrolysate (Bhojwani, 1968; Nag, 1970).

Interestingly, the endosperm of grasses (rye-grass and maize) do not require auxin for growth in vitro (Strauss and LaRue, 1954; Norstog, 1955; Tamaoki and Ullstrup, 1958). However, in the studies cited, yeast extract, tomato extract, or coconut milk were required for growth and though the absence of requirement for exogenous auxin appears clear-cut, the possibility remains that such incompletely defined natural substances may in some way satisfy an auxin requirement. Two studies, that of Strauss (1960) and the present one, clearly demonstrate that maize and rye-grass endosperm are completely autonomous with respect to auxin. Either they do not require any auxin or they supply their own. The latter interpretation is consistent with our understanding of the growth of autonomous tissue lines in culture (Braun, 1954).

Bhojwani (1968) has shown that mature endosperm of *Croton* and *Osyris* proliferated in vitro of 2, 4-D—containing media so long as the embryo was also included, and that the requirement for presence of embryo could be replaced by the addition of gibberellic acid. He suggested that gibberellic acid might be able to induce proliferation of plant endosperms generally. However, it is completely ineffective in inducing callus formation in barley endosperm and has no stimulatory effect on rye-grass endosperm. Moreover, Nag (1970) was unable to demonstrate a relation between embryo and endosperm callusing in several parasitic dicotyledons. In his studies, subculturable callus was obtained from mature endosperms of *Dendrophthoe, Leptomeria* and *Taxillus* with media containing kinetin (0.5—10 mg/l) and indole butyric acid (2—40 mg/l). Similar results were obtained by Rangaswamy and Rao (1963), and Satsangi and Mohan Ram (1965) in endosperm cultures of *Santalum* and *Ricinus* respectively, and *Cucumis* endosperm has also been grown in culture by Nakajima (cited in Rangaswamy and Rao, 1963) and shown to have a requirement for exogenous auxin. Higher kinetin levels (around 5 mg/l) and lower auxin levels (around 2 mg/l IAA) resulted in formation of adventitious buds in endosperm of *Dendrophthoe, Leptomeria* and *Taxillus,* (Nag, 1970). Endosperm buds and roots have also been reported in cultures of *Croton* and *Exocarpus* (Bhojwani, 1968).

Grass embryos, as represented by barley, do not require exogenous auxin for normal development. Medium J meets all of the requirements for essentially normal growth of proembryos (see Fig. 4). However, this medium is incapable of sustaining callus production by embryonic barley tissues unless relatively high concentrations of 2, 4—D (10 mg/l) are added to the medium. Thus a clear-cut difference exists between grass endosperm (rye-grass, maize) and embryos (barley) regarding an auxin requirement for sustained callus proliferation. It is possible to generalize somewhat at this point. Certain tissue cultures derived from plant tumors are characterized by their independence of an exogenous supply of auxin (Kehr and Smith, 1953; Braun, 1954). In this sense, auxin-independent endosperm cultures behave like plant tumors. Moreover, they also exhibit certain chromosomal abnormalities associated with tumors (Strauss, 1954; Norstog, 1956b). Furthermore, auxin-independent endosperm tissue cultures have not been observed to undergo organogenesis in vitro (Norstog, et. al., 1969; Strauss, 1954) except for an isolated instance reported by LaRue (1947) in maize endosperm cultures, but not susbsequently encountered (Strauss, 1954). Except for the report by Lampton (1953) that the endosperm of *Asimina* proliferated on auxin-free (but not defined) media, endosperms of dicotyledons seem to require exogenous auxin for growth in vitro. Thus one can say that in general the endosperms of monocotyledons differ from dicotyledons with respect to their auxin requirement as well as in their ability to undergo organogenesis in vitro. The exception to this, the endosperm of *Asimina* did not undergo organogenesis in vitro, differentiation being limited to some minor differences in cell morphology in certain experimental cultures (Lampton, 1954). One can state, theorefore, that those endosperms that require exogenous auxin for growth in vitro also are likely to develop roots and shoots, in contrast to the auxin-independent lines. Whether or not, in addition, the fact that the auxin-independent endosperm cultures were obtained from developing endosperm, whereas the auxin-dependent lines were derived from mature endosperms, is significant, cannot be stated at this time. However, the point is an important one and certainly suggests the need for additional exploration of the requirements of developing vs. mature endosperms in vitro.

In conclusion, endosperm and embryo development differ in terms of the structural components of their respective cells, in apparent biosynthetic activity of those cells, in their requirements for growth in vitro and, in particular, their dependence or independence of exogenous auxin. Although the biochemical, cytological, morphological, and morphogenetic characteristics of the two systems are becoming increasingly well-defined, the question remains as to the fundamental cause(s) of their distinctively differing patterns of growth.

References

Bhojwani, S. S. 1968. *Morphogenetic Studies on Cultured Mature Endosperm and Embryo of Some Angiosperms*. Ph.D. thesis, Univ. of Delhi, Delhi, India.

Blakely, L. M., and F. C. Steward. 1964. Growth and organized development of cultured cells V. The growth of colonies from free cells on nutrient agar. *Am. J. Bot.* **51**: 780-791.

Braun, A. C. 1954. The physiology of plant tumors. *A. Rev. Pl. Physiol.* **5**: 133-162.

Brink, R. A., and D. C. Cooper. 1947. The endosperm in seed development. *Bot. Rev.* **13**: 423-541.

Chibnall, A. C. 1939. *Protein Metabolism in Plants*. Yale Univ. Press, New Haven, Conn.

Diboll, A. G. 1968. Fine structural development of the megagametophyte of *Zea mays* following fertilization. *Am. J. Bot.* **55**: 787-806.

——, and D. A. Larson. 1966. An electron microscopic study of the mature megagametophyte in *Zea mays*. *Am. J. Bot.* **53**: 391-402.

Filner, P. 1966. Regulation of nitrate reductase in cultured tobacco cells. *Biochem. Biophys. Acta.* **118**: 299-310.

Jensen, W. A. 1965. The ultrastructure and composition of the egg and central cell of cotton. *Am. J. Bot.* **52**: 781-787.

Kehr, A. E., and H. H. Smith. 1953. Genetic tumors in *Nicotiana* hybrids. *Brookhaven Symp. Biol.* **6**: 55-78.

Lampton, R. K. 1953. Developmental and experimental morphology of the ovule and seed of *Asimina triloba* Dunal. *Diss. Abst.* **13**: 290-291.

LaRue, C. D. 1947. Growth and regeneration of the endosperm of maize in culture. *Am. J. Bot.* **34**: 585-586.

Linsmaier, E., and F. Skoog. 1965. Organic growth factor requirements of tobacco tissue cultures. *Physiologia Plantarum* **18**: 100-127.

Maheshwari, P. 1950. *An Introduction to the Embryology of Angiosperms*. McGraw-Hill Book Co., Inc., New York, N. Y.

Nag, K. K. 1970. *Morphogenetic Studies on Endosperm, Embryo and Other Sporophytic Tissues of some Parasitic Angiosperms*. Ph.D. thesis, Univ. of Delhi, Delhi, India.

Norstog, K. 1955. *The Culture of Excised Ovules, Endosperms and Embryos of Certain Grasses*. Ph.D. thesis, Univ. of Michigan Ann Arbor, Mich.

——. 1956a. The growth of barley embryos on coconut milk media. *Bull. Torrey bot. Club.* **83**: 27-29.

——. 1956b. Growth of rye-grass endosperm *in vitro*. *Bot. Gaz.* **117**: 253-259.

——. 1961. The growth and differentiation of cultured barley embryos. *Am. J. Bot.* **48**: 876-884.

——. 1967. Studies on the survival of very small barley embryos in culture. *Bull. Torrey bot. Club* **94**: 223-229.

——, and J. E. Smith. 1963. Culture of small barley embryos on defined media. *Science* **142**: 1655-1656.

——, W. E. Wall, and G. P. Howland. 1969. Cytological characteristics of ten-year-old rye-grass endosperm tissue cultures. *Bot. Gaz.* **130**: 83-86.

Rangaswamy, N. S., and P. S. Rao. 1963. Experimental studies on *Santalum album* L. —Establishment of tissue culture of endosperm. *Phytomorphology* **13**: 450-454.

Satsangi, A., and A. Y. Mohan Ram. 1965. A continuously growing tissue culture from the mature endosperm of *Ricinus communis* L. *Phytomorphology* **15**: 26-30.

Strauss, J. 1954. Maize endosperm tissue grown *in vitro* II. Morphology and cytology. *Am. J. Bot.* **41**: 833-839.

——. 1960. Maize endosperm tissue grown *in vitro* III. Development of a synthetic medium. *Am. J. Bot.* **47**: 641-647.

——, and C. D. LaRue. 1954. Maize endosperm tissue grown *in vitro* I. Culture requirements. *Am. J. Bot.* **41**: 687-694.

Steward, F. C., L. M. Blakely, A. E. Kent, and M. O. Mapes. 1963. Growth and organization in free cell cultures. *Brookhaven Symp. Biol.* **16**: 73-88.

Tamaoki, T., and A. J. Ullstrup. 1958. Cultivation *in vitro* of excised endosperm and meristematic tissues of corn. *Bull. Torrey bot. Club.* **85**: 260-272

Tulecke, W., L. H. Weinstein, A. Rutner, and H. J. Laurencot, Jr. 1961. The biochemical composition of coconut water (coconut milk) as related to its use in plant tissue culture. *Contrib. Boyce Thompson Inst.* **27**: 115-128.

Whaley, W. G., and H. H. Mollenhauer. 1963. The Golgi apparatus and cell plate formation—a postulate. *J. Cell. Biol.* **17**: 216-221.

White, P. R. 1943. *A Handbook of Plant Tissue Culture*. Ronald Press, New York, N. Y.

Ziebur, N. K., and R. A. Brink. 1951. The stimulative effect of *Hordeum* endosperms on the growth of immature plant embryos *in vitro*. *Am. J. Bot.* **38**: 253-256.

Adv. Pl. Morph. **1972: 126-132**

APOMIXIS

By Gwenda L. Davis

Department of Botany, University of New England, Armidale, N.S.W. 2351, *Australia*

ABSTRACT

The three commonest forms of apomixis, aneuspory, somatic apospory and gonial apospory, are described briefly and the occurrence of multiple embryo sacs in somatic apospory is discussed. When these develop in close association, their bounding membranes are indistinct and, when each contains an embryo, there is a superficial resemblance to a single embryo sac containing several embryos. It is suggested that this is the explanation of the so-called endosperm and antipodal embryos described by some authors. Attention is drawn to the almost complete lack of detailed information on the events which occur in the anthers of apomicts. Although apomyxis was once considered to be a reproductive aberration of no importance to the species, it is more accurately described as a sophisticated form of vegetative reproduction and is probably more common and widely spread among angiosperms than the records suggest.

INTRODUCTION

The formation of viable seeds in the absence of fertilization was reported by several workers during the 19th century (see Gustafsson, 1946) but it was not until 1908 that Winkler coined the term *apomixis* to describe the substitution of an asexual process for the usual sexual phenomena.

Investigations into the origin of such embryos soon showed that they could originate in several different ways and a more precise terminology was required to describe the different apomictic processes operating.

To meet this need, apomixis was reviewed and special terms were advanced by Gustafsson (1935, 1944, 1946 & 1947), Fagerlind (1940, 1944), Stebbins (1941), Battaglia (1947) and others. Inevitably, many of these terms were controversial or lacking in exact meaning, but Battaglia (1963) brought order into what was becoming a chaotic state of affairs and it is his terminology which is followed in the present paper.

Whereas previous workers had followed Winkler (1908) in including under apomixis, those instances where sporophytic tissue invades the sexually formed embryo sac and develops into embryos *in situ*, Battaglia (1947, 1963) excluded these as instances of *sporophytic embryony*. He then restricted the term apomixis to those instances in which the embryo develops autonomously from a cell of the embryo sac itself, whether this structure is reduced or unreduced in its chromosome complement.

Not a great deal is known about apomixis in reduced embryo sacs and most of the examples cited by Battaglia (1963) are drawn from hybrids or following experimental treatment.

The commonest manifestations of apomixis under natural conditions occur in connection with unreduced embryo sacs and, of these, *aneuspory*, *gonial apospory* and *somatic apospory* are naturally occurring phenomena which may be facultative or obligate. Of the first two, aneuspory and gonial apospory, the mechanism of apomixis is based on the partial or complete failure of meiosis and the 2n embryo sac is formed from the megaspore mother cell, without the formation of megaspores.

ANEUSPORY

This was first reported in *Taraxacum officinale* (Juel, 1904, 1905), where it is the only method of seed formation, but it has since been found to be characteristic of other polyploid species. Other reported examples listed by Battaglia (1963) are species of *Balanophora, Antennaria, Chondrilla, Hieracium, Wikstroemia* and *Elatostemma* but, in these, a connection with polyploidy has not been investigated.

Of the eight types of aneuspory recognised by Battaglia, the best known is the *Taraxacum type* and, in this, a restitution nucleus is formed after meiosis I. Cytokinesis follows meiosis II and the chalazal dyad cell develops into a 2n embryo sac in which the reduced egg gives rise directly to the embryo.

GONIAL APOSPORY

Meiosis does not occur. The megaspore mother cell enlarges, a small vacuole appears above and below its nucleus, and the cell becomes the 1-nucleate embryo sac. The three successive nuclear divisions are mitotic and the resulting 8-nucleate embryo sac (2n) is indistinguishable from one of sexual origin. In *Brachycome* (Davis, 1964a, 1967), the polar nuclei function directly as the first two endosperm nuclei and the derived endosperm is therefore, 2n. Similar behaviour has been reported in *Antennaria alpina* (Juel, 1900), *Alchemilla alpina* (Murbeck, 1901) and *Eupatorium glandulosum* (Holmgren, 1919) although, in the last species, there is occasional formation of a secondary nucleus. In *B. ciliaris* both embryogeny and endosperm formation are initiated in the bud and, at anthesis, the embryos are at the 3rd or 4th cell generation and surrounded by endosperm.

SOMATIC APOSPORY

The mechanism of somatic apospory differs from that of both aneuspory and gonial apospory in that meiosis is apparently normal but the megaspores play no part in embryo sac formation. Either during or soon after meiosis one or more sporophytic cells enlarge and invade the nucellar lobe, destroying and replacing the megaspores. After three successive nuclear divisions, each such cell becomes differentiated into an aposporic 2n embryo sac with the "normal" organization. In *Poa pratensis* (Kiellander, 1941) and in about 10% of the ovules of *Calotis lappulacea* (Davis, 1968) the megaspore mother cell is destroyed before it enters meiosis.

The time of initiation of the aposporic embryo sacs does not appear to be related to the stage of development of the ovules or the position of the flowers in an inflorescence. This is particularly apparent in the Compositae, where their formation bears no relationship to the centripetal development characteristic of sexual species. During early embryogeny, embryos at widely differing stages of development occur at random across the capitulum.

Multiple embryo sac formation is common in connection with somatic apospory, but the numbers of cells which invade the nucellar lobe is probably determined by space considerations. Up to three such embryo sacs have been found in *Minuria integerrima* (Davis, 1964b) and *Calotis lappulacea* (Davis, 1968) but others may deveolop in situ within the chalaza. It is these chalazal aposporic embryo sacs which have been described as "aggressive antipodal haustoria" in *Artemisia tridentata* (Diettert, 1938), *Grindelia squarrosa, Gutierrezia* sp. and *Haplopappus ciliatus* (Howe, 1926, 1959). In *Calotis lappulacea* (Davis, 1967, 1968) such enlarging embryo sacs sometimes penetrate the ovular epidermis and extend into the loculus of the ovary where they ultimately collapse.

Endosperm formation, usually without preceding fusion of polar nuclei, occurs only in those embryo sacs which occupy the "normal" site, but the unreduced egg of each may give rise to an embryo. For this reason, the occurrence of more than one embryo in an ovule (false polyembryony) is associated with multiple embryo sac formation and the positions occupied by the embryos is determined by the positions of their embryo sacs. When the boundaries between closely associated embryo sacs are overlooked, appearances may suggest the presence of two or more embryos in different positions within a single structure. This has led to the claim that endosperm cells or, even, antipodals may give rise to embryos.

In each example of this type of polyembryony in *Minuria integerrima* (Davis, 1964b), the embryo sac closest to the micropyle contains the more advanced embryo but no endosperm, whereas normal endosperm was present in the more deeply located embryo sacs. Haberlandt (1921) figured an ovule of *Hieracium aurantiacum* containing what he described as a typical (i.e., sexual) and an aposporic embryo sac but, while the former contained a large secondary nucleus, endosperm as well as an embryo was present in the latter. It is probable that both these embryo sacs were aposporic and there is no record of a megaspore ever developing after somatic apospory has been initiated in an ovule.

ANTHERS OF APOMICTS

Most investigations on apomixis have been concerned with events in the ovules and little or no attention has been paid to the anthers, although there are occasional references to the occurrence of plasmodial microspore mother cells, polyads and other abnormalities.

Because both aneuspory and gonial apospory are based on either modification or suppression of meiosis in the megaspore mother cell, it is a reasonable assumption that parallel events will occur in the microspore mother cells. However, in *Erigeron karwinskianus* var. *mucronatus* although Battaglia (1950) found aneuspory in 80% of the ovules and Polygonum type of embryo sac development in the remainder, he reported that in the anthers of both types of floret "the general course of meiosis is regular, the result being generally, normal tetrads". This suggests that meiosis in the two sites is controlled independently.

In connection with gonial apospory, there is even less information. In *Brachycome ciliaris* (Davis, 1964a), failure of meiosis in the microspore mother cells results in the formation of plasmodial 2-or 4-nucleate spore mother cells, polyads and irregularly shaped 2-nucleate pollen grains.

Species exhibiting somatic apospory show great variation in the events which occur in their anthers. In their work on *Crepis* spp., Stebbins and Jenkins (1939) found no correlation between the behaviour of the spore mother cells of the ovule and anther and they concluded that independent genetic factors operated in the two sites. The same appears to be true in *Panicum maximum* (Warmke, 1954) and *Paspalum secans* (Snyder, 1957) where normal n pollen is formed and germinates on the stigmas. On the other hand, the embryo sac is unreduced and, while the "egg" develops autonomously into an embryo, the 4n secondary nucleus requires fertilization by a male gamete before giving rise to endosperm. A similar tendency to different behaviour in the two sites is seen in *Minuria integerrima* (Davis, 1964b) and *Calotis lappulacea* (Davis, 1968). In both these species there is regular meiosis in the megaspore mother cells but, in the microspore cells, lagging chromosomes at anaphase 11 are common and lead to the formation of micronuclei. Only two microspores of each tetrad are usually of normal size and staining reaction but the main disturbance lies in gametogenesis. The generative nucleus rarely divides and, consequently, the pollen remains at the 2-celled stage when shed in contrast to the 3-celled condition in sexual Compositae. An interesting phenomenon occurs in the anthers of *C. lappulacea* (Davis, 1968) where certain tapetal cells enlarge during microsporogenesis and become plasmodial, often filling the loculus and crushing the tetrads. Such behaviour has not been reported elsewhere, although the embryo sac-like pollen grains of the apomict *Leptomeria billardieri* (Ram, 1959) may well prove to be tapetal rather than sporogenous in nature.

TERMINOLOGY OF THE APOMICTIC EMBRYO SAC

The unreduced apomictic embryo sac is to be regarded as a specialized sporophytic structure which exhibits remarkable morphological convergence to its reduced sexual counterpart and fulfils the same function.

However, because embryological terms are defined on the assumption that sexual reproduction and alternation of generations operate, they are not strictly applicable to any apomictic structures. For example, by definition the embryo sac is the female gametophyte and its nuclei have the reduced or n chromosome complement. In an apomict, however, the analogous structure is sporophytic (2n) and does not produce gametes. Similarly, if the egg be defined as the female gamete, the morphologically similar but 2n cell in the apomictic embryo sac should not receive the same name because, although it gives rise to the embryo, it does so without fertilization. There is clearly no equivalent to the zygote in an apomict, and even the use of the term embryo is not strictly applicable although in its formation, the unreduced egg undergoes the same sequence of cleavages as occur in the zygote of related sexual species.

One solution to this problem is the adoption of a special terminology for apomicts but, in practice, this procedure would probably not be acceptable. The alternative, and that adopted in the present paper, is to retain the sexually based terms but to qualify them with such epithets as "unreduced", "non-reduced" or "apomictic". While this procedure amounts to a contradiction in terms, at least there is no ambiguity in meaning.

OCCURRENCE OF APOMIXIS

Apomixis have been reported in relatively few families of angiosperms (see Battaglia, 1963) but its rarity is probably more apparent than real.

One reason for this is that many authors refrain from reporting instances in which occasional ovules appear to exhibit abberations in megasporogenesis and embryo sac formation, and overlook the operation of facultative apomixis.

Another reason is the common practice of basing embryological investigations on insufficient material drawn from only one source or, even, a single plant. It is important to bear in mind that details of reproduction are subject to variation in the same way as traditional taxonomic characters. Just as a taxonomist bases his concept of a species on plants drawn from populations throughout its whole range, so an embryologist should obtain his material from varying ecological situations and widely spaced localities. It is only in this way that the reproductive behaviour of a species can be understood and the occasional operation of apomixis detected. It is probably no accident that the families in which apomixis is said to be commonest (Compositae, Gramineae) are those in which the greatest number of investigations have been made (Davis, 1966).

THE CAUSES OF APOMIXIS

Whatever evidence there is, indicates that apomixis is inherited genetically. In *Bothriochloa* and *Dichanthium*, Harlan, Brooks, Borgaonkar and de Wet (1964) found it to be dominant to but independent of sexuality. The two were not alternative modes of reproduction but were independent phenomena and the genes controlling sexual reproduction were non-allelic to those controlling apomixis. If this is true generally, then any line of descent which carries the genes for apomixis will produce apomicts as well as sexual plants. In other words, the species will exhibit facultative apomixis. In certain circumstances, however, the expression of the genotype may be influenced by internal or external factors. Nygren (1949) reported that in *Calamagrostis purpurea* the first formed panicles are sexual but, on ageing, the same plants became apomictic. The environment was shown to be important in *Limònium transwallianum* in which Hjelmqvist and Grazi (1964) found an increase in the proportion of apomictic embryo sacs when plants were grown in a warm glasshouse, as compared to those grown under cool conditions outside.

The connection between aneuspory and polyploidy in *Taraxacum* is well established and there are similar reports with apospory in the Gramineae. For example, in *Bothriochloa* and *Dichanthium* (Harlan, Brooks, Borgaonkar and de Wet, 1967) diploid species reproduce sexually and their polyploids are facultative apomicts, while in *Tripsacum dactyloides* (Farquharson, 1955) apomixis only occurs in the 4n races.

APOMIXIS AND EVOLUTION

Inherent in sexual reproduction is the mechanism which initiates and maintains variability within the species. Such potential variability can be expressed as a normal curve, from one section or another of which the environment selects the breeding population of the next generation. Implicit in this, is the failure to survive of all variants equipped with genotypes unsuitable to that particular environment. Should the environment change, however, selection will operate in favour of individuals equipped with different genotypes, which are drawn from another section of the n-curve of potential variability. Such a mechanism ensures the survival of the species under changing external conditions and may lead to the formation of ecotypes.

Apomixis, however, represents a different mechanism and one whose effects are opposite to those of sexual reproduction in that variability is "frozen" and offspring have the same genotype as the parent plant.

The question to be examined is, whether the operation of apomixis has an adverse effect on the survival of the species. For facultative apomicts, the best comparison is with species in which sexual reproduction is supplemented by vegetative reproduction. Such plants seem to have acquired the best of both worlds in that variability is stored up in the species as a whole but its survival does not depend on the formation of seeds by sexual methods, with all the inherent hazards such as availability of pollen vectors and so on. Provided the environment is stable, vegetative reproduction is a highly successful method of perpetuating the species. Its disadvantage, however, lies in its failure as a means of distribution over anything but a very limited area, whereas seeds are adapted for wide dissemination by a variety of agents.

Returning to apomixis, we find that this very specialized form of vegetative reproduction has "taken over" the sexual means of distribution. In other words, a small portion of the parent sporophyte, the apomictic embryo, becomes enclosed within the seed which is indistinguishable from the same structure produced by sexual means.

Apomixis, then, is merely a sophisticated form of vegetative reproduction and facultative apomicts bear the same relationship to evolution as sexually reproducing plants with ancillary reproduction by vegetative means.

Facultative apomixis is very common in grasses, where an equilibrium appears to have been established with sexual reproduction. Both processes operate concurrently and investigations suggest that the apomictic component of a population is usually more fertile and vigorous than the sexual. In the Panicoideae of the Gramineae, Brown and Emery (1958) investigated 164 species in 64 genera of 16 tribes and they found apomixis in 28% of the species. They concluded from this that apomixis has been a characteristic of the subfamily since soon after its divergence from other Gramineae and before its diversification into its component tribes.

In the case of obligate apomixis, the analogy is with those species which have lost the ability to reproduce sexually and which survive only by virtue of vegetative reproduction. Such species represent an evolutionary dead-end, because they no longer possess the capacity to produce genetic variants or hybrids.

REFERENCES

BATTAGLIA, E. 1947. Sulla terminologia dei processi apomittici. *Nuovo bot. ital.* **54**: 674-696.

——. 1950. L'alterazione della meiosi nella riproduzione apomittica di *Erigeron karwinskianus* var. *mucronatus*. *Caryologia* **11**: 165-204.

——. 1963. Apomixis. In *Recent Advances in the Embryology of Angiosperms*. Maheshwari, P. (ed.). Int. Soc. Pl. Morphologists, Delhi. 221-264.

BROWN, W. V., AND W. H. P. EMERY. 1957. Apomixis in the Gramineae. *Am. J. Bot.* **45**: 253-263.

DAVIS, G. L. 1964a. Embryological studies in the Compositae. 4. Sporogenesis, gametogenesis and embryogeny in *Brachycome ciliaris*. *Aust. J. Bot.* **12**: 142-151.

——. 1964b. The embryology of *Minuria integerrima*—a somatic apomict. *Phytomorphology* **14**: 231-239.

——. 1966. *The Systematic Embryology of Angiosperms*. John Wiley, New York.

——. 1967. Apomixis in the Compositae. *Phytomorphology* **17**: 270-277.

——. 1968. Apomixis and abnormal anther development in *Calotis lappulacea*. *Aust. J. Bot.* **16**: 1-17.

DIETTERT, R. A. 1938. The morphology of *Artemisia tridentata*. *Lloydia* **1**: 3-74.

FAGERLIND F. 1940. Die Terminologie de Apomixis Prozesse. *Hereditas* **26**: 1-22.

——. 1944. Is my terminology of the apomictic phenomena of 1940 incorrect and inappropriate ? *Hereditas* **30**: 590-596.

FARQUHARSON, L. I. 1955. Apomixis and polyembryony in *Tripsacum dactyloides*. *Am. J. Bot.* **42:** 737-743.

Gustafsson, A. 1935. Studies on the mechanism of parthenogenesis. *Hereditas* **21:** 1-112.

——. 1944. The terminology of apomictic phenomena. *Hereditas* **30:** 145-151.

——. 1946/1947. Apomixis in higher plants. 1-111. *Acta Univ. Lund* **42,** 43: 1-370.

Haberlandt, G. 1921. Die Entwicklungserregung der Eizellen einiger parthenogenetischer Kompositen. *Sber. preuss. Akad. Wiss.* **51:** 861-881.

Harlan, J. R., H. M. Brooks, D. S. Borgaonkar, and J. M. J. de Wet. 1964. Nature and inheritance of apomixis in *Bothriochloa* and *Dichanthium*. *Bot. Gaz.* **125:** 41-46.

Hjelmqvist, H., and F. Grazi. 1964. Studies on variation in embryo sac development. *Bot. Notiser* 1964: 141-166.

Holmgren, I. 1919. Zytologische Studien über die Fortpflanzung bei den Gattungen *Erigeron* und *Eupatorium*. *K. Svenska Vat.-Akad. Handl.* **59:** 3-118.

Howe, T. D. 1926. Development of the embryo sac in *Grindelia squarrosa*. *Bot. Gaz.* **81:** 280-296.

Howe, T. D. 1959. Recent studies of the female gametophyte in the Compositae, especially in the tribe Asterae. *Proc. IX. Intern. bot. Congr., Montreal* **2:** 171-172.

Juel, H. O. 1900. Vergleichende Untersuchungen über typische und parthenogenetische Fortpflanzung bei der Gattung *Antennaria*. *K. Svenska Vet.-Akad. Handl.* **33:** 1-59.

——. 1904. Die Tetradenteilungen in der Samenanlage von *Taraxacum*. *Ark. Bot.* **2:** 1-9.

——. 1905. Die Tetradenteilungen bei *Taraxacum* und anderen Cichorieen. *K. Svenska Vet.-Akad. Handl.* **39:** 1-21.

Kiellander, C. L. 1941. Studies in apospory in *Poa pratensis*. *Svenska bot. Tidskr.* **35:** 221-332.

Murbeck, S. 1901. Parthenogenetische Embryobildung in der Gattung *Alchemilla*. *Acta. Univ. Lund* (2) **36:** 1-41.

Nygren, A. 1949. Apomictic and sexual reproduction in *Calamagrostis purpurea*. *Hereditas* **35:** 285-300.

Ram, M. 1959. Occurrence of embryo sac-like structures in the microsporangia of *Leptomeria billardieri*. *Nature, Lond.* **184:** 914-915.

Snyder, L. A. 1957. Apomixis in *Paspalum secans*. *Am. J. Bot.* **44:** 318-324.

Stebbins, G. L. 1941. Apomixis in the Angiosperms. *Bot. Rev.* **7:** 507-542.

Stebbins, G. L., and J. A. Jenkins. 1939. Aposporic development in the North American species of *Crepis*. *Genetica* **21:** 191-224.

Warmke, H. E., 1954. Apomixis in *Panicum maximum*. *Am. J. Bot.* **41:**5-11.

Winkler, H. 1908. Über Parthenogenesis und Apogamie in Pflanzenreich. *Progr. Rei. Bot.* **2:** 294-454.

Adv. Pl. Morph. 1972: 133-136

RECENT FINDS OF SOME RARE ANGIOSPERMS IN WESTERN HIMALAYA AND THEIR PHYTOGEOGRAPHIC SIGNIFICANCE[1]

M. A. RAU

Botanical Survey of India, Northern Circle, Dehradun, India

ABSTRACT

The paper records the finding of some interesting plants with a known eastern distribution, for the first time, in western Himalaya. The phytogeographic significance of the occurrence of such plants in stations widely separated from the previously known ones is discussed. A plea is made for more intensive collections in the western Himalayan sectors in view of the promise held out by these recent discoveries.

Recent collections in various sectors of the western Himalaya have brought to light the occurrence of some rare species of Angiosperms which were previously known only from localities in Sikkim and further east or which were considered to have a very restricted distribution. Such finds in new localities far removed from their previously known stations are always of interest and raise certain phytogeographical problems. Among such recent finds made in western Himalaya by the author and his colleagues are some of the species which were till now regarded as belonging exclusively to the eastern Himalayan floristic province or some which were considered to be strictly endemic in Sikkim. It is proposed to discuss in this brief paper some of these new distributional records and their significance.

Didiciea cunninghamii King & Prain and *Listera longicaulis* King & Pantling, are two terrestrial orchids known from the Lachen Valley in Sikkim, having been collected there during the closing years of the last century and till now regarded as being confined to Sikkim alone. Our collection of these two species in the Gangahrea Forest of Garhwal Himalaya, nearly 1000 km west of Sikkim is, therefore, of interests.

Cypripedium elegans Reichb. f., a slipper orchid, characterised by the possession of a single pair of opposite leaves, has always been considered to have an eastern distribution with its known occurrence in Sikkim, Yunnan and a few other localities in China. This orchid was recently collected in the Garhwal Himalaya at an altitude of 3300 m, extending its distribution thus far west. Some years ago it was collected in Nepal.

Bulbophyllum triste Reichb. f., an epiphytic orchid originally collected on Mt. Toungoo in Tenasserim was later found in the Teesta Valley in Sikkim. It has now been collected in the subtropical zone of the Kumaon Himalaya.

Bulbophyllum densiflorum Rolfe of Bhutan, *Cirrhopetalum cornutum* Lindl. and *Oberonia myriantha* Lindl. of Sikkim and Assam and *Pleione grandiflorum* Rolfe of Yunnan have also been collected for the first time in west Himalayan stations.

---

[1] Based on a paper presented to the Symposium on Recent Advances in the Taxonomy of Angiosperms held during the Indian Science Congress Session in Chandigarh. 1966.

The above instances relate to the more westward extension of the known range of distribution of what were hither to regarded as the east Himalayan elements. There are also some recent finds which provide examples of an eastward extension of the westward elements. Among these are, again, two orchids which were till recently recorded only from Kashmir in our country, one of them widespread in North America and Europe and the other known only from two collections in Kashmir. *Corallorhiza trifida* Chatel, a peculiar terrestrial orchid, known in North America as the 'Northern Coral Root', enjoys a wide distribution occurring in boggy areas, in deep moss on coniferous forest slopes, in Cedar, Tamarack or Spruce swamps (Correll, 1950). We have now collected this orchid in the alpine swampy zone in Kumaon Himalaya thus giving a it more eastward distribution in the Himalaya. *Epipogium tuberosum* Duthie was till very recently considered to be confined to Kashmir but the present writer has collected this peculiar saprophytic species not only in the Kulu Himalaya but further east in Garhwal Himalaya, in both cases, the orchid appearing in the same type of environment, in the undergrowth of a fir-spruce forest.

We have so far considered the above new finds all of which belong to the family, Orchidaceae. The recent find from two new localities in Garhwal Himalaya of the extremely interesting botanical curiosity, *Circaeaster agrestis* Maxim, deserves mention. This plant was collected for the first time in Kumaon by Strachey and Winterbottom during the middle of the last century and similar specimens gathered in China and sent to Leningrad were named by Maximowicz, as *Circaeaster agrestis*. The specimens collected by Strachey and Winterbottom were, however, lost and it has been recorded (see Foster 1963, for the botanical history and morphology of this curious plant) that Hooker wrote to Duthie, who was on a collection trip to the Himalaya, that "the plant is worth a pilgrimage for I know nothing in the least like it". Duthie did succeed in rediscovering this species in Kumaon and, till very recently, this was the only extant collection from the west Himalaya, though it was known from many localities in the east Himalaya and in China. We have succeeded recently in collecting this plant from not only the original collecting locality in Kumaon but also from two more stations in Garhwal Himalaya thus extending the known distribution further westward to the extent of about 100 km.

Another interesting find may also be mentioned here. The collections of Strachey and Winterbottom included a Scrophulariaceous herb which had also been collected earlier by Falconer. This was more than a hundred years ago and the plant was named after Falconer by Hooker as a new genus, *Falconeria* (since brought under *Wulfenia* by Pennell 1943). It was not collected again for more than a hundred years till we found it in the same Kumaon Valley. The significance of the find lies in the fact that the species has neither extended its range nor has become extinct.

Rhododendron nivale Hook. f. and *Hydrobryum griffithii* (Wall.) Tulasne are other recent additions to the west Himalayan flora. The latter, a Podostemaceous member, previously recorded from eastern Nepal, Sikkim, Assam, Burma, Thailand and China, was surprisingly met with in a stream in Garhwal Himalaya. Some years earlier a British Museum

Expedition had found it in the Jumla area of west Nepal.

The foregoing account of some of the new distribution records emphasises the need for further intensive exploration and also the need for the study of the environment and biology of the species concerned. In the absence of precise data on the extent of distribution, the phylogenetic history, biological factors like insect visitors, seed dispersal methods and floristic associations, it is difficult to assess the phytogeographical significance except to the extent of revealing the present range and extent of distribution. The occurrence in abundance of the botanically puzzling, *Circaeaster agrestis* in two new localities indicates that the species is by no means rare in the area and could be expected to occur in localities with a suitable environment. van Steenis (1934), in a study of the Malaysian mountain flora, has referred to certain features of plant geography that no species is homogeneously distributed throughout the area it occupies and that floristic discontinuity is bound up with environmental discontinuity. In the case of *Circaeaster*, the plant appears to have a wide range as well as adaptability, judging by the fact that it has been successfully cultivated in places like Kew, Edinburgh and Berkeley besides Dehra Dun where it has flowered and produced viable seeds. On the other hand, the rare *Falconeria himalaica* (= *Wulfenia himalaica*) does not appear to have spread, as it is not known from any other locality though Pennell hinted at the possibility of its occurring in Nepal. There is no record of any such collection as far as is known to us.

The finding of the rare *Epipogium tuberosum* from localities in Kulu and Garhwal thus giving it a range of distribution from Kashmir, its type locality, to Garhwal is of some interest. It is known that another species of this genus, *E. aphyllum*, which is widely distributed, from western Europe to Japan, has a similar range of distribution in western Himalaya, both the species occurring together in Garhwal. Referring to yet another widely distributed species of the genus, *E. roseum*, Holttum (1957) has remarked "it is also remarkable that some more exacting species are equally widespread, e.g., the interesting saprophyte, *Epipogium roseum*, only once found in Malaya and certainly not common but distributed from Africa to Australia."

In the case of epiphytic orchids it is well known that there is a great and continuous region suitable for these orchids from the eastern Himalaya southwards to Malaya and Java and eastwards to the Philippines and New Guinea. In the Sikkim Himalaya there are many genera which also occur in Java or in New Guinea (Holttum, 1957). In the western Himalaya, while the terrestrial species are found throughout the range, the epiphytic species gradually fade out west of Tehri-Garhwal. In some of the lower valleys of Garhwal and Kumaon, however, many epiphytic species are met with and in some places, the orchid flora is as rich as in any of the east Himalayan localities. Many of the genera and species are common to both and it can, therefore, be safely concluded that the rich epiphytic orchid belt of the east Himalaya and southward may actually have its western limit in the Garhwal and Kumaon Valleys of western Himalaya. The main determining factor in this distribution is the climatic one with localities having a fairly high rainfall and a pronounced dry season showing a large proportion of such orchids (Holttum 1957). It is also well recognized that the Sumatran Track and Luzon Track, well known in Malaysian Phytogeography, have their connection with the Himalayan highlands. Some of the Himalayan species are found in one or the other of these tracks and a few in both. Among the species thus represented in the Himalaya as well as in one or the other or both Malaysian tracks are, *Boenninghausenia albiflora, Drosera peltata, Geranium nepalense, Hemiphragma heterophyllum, Myriactis nepalensis* and *Sarcococca saligna*. In this connection, the interesting distribution of *Viscum loranthii* Elmer may be mentioned. This parasite is found in Sumatra as well as in the Philippines. It was recorded from localities in Garhwal and Tehri-Garhwal by collectors early in this century. This was surprising in view of the wide gap existing between the southern and northern limits but it has since been found in an intermediate station in Manipur. In all these localities, this curious species occurs as a parasite on another Loranthaceous species, in particular, on *Taxillus vestitus* in the western Himalaya. This is by no means common in this area though we have collected it recently from a new station in Kumaon.

The above aeccount has thus highlighted the significance of some of the recent finds and serves to emphasise the need for a intensive work in the field of floristical Plant Geography. van Steenis (1934) states "Taxonomy and floristical Plant Geography are basic branches of botanical science. The taxonomist and the plant geographer need study in the fields as well as in the herbarium and laboratory. He ought to study his material morphologically and cytologically both in the original spot and in cultivation in order to get an insight into the range of its variability and alliance. He ought to study the ecological claims and tolerance and the sociological standing of his plants. The whole working scheme must remain the theoretical ideal which in practice will be reached in the case of a few plant species only".

While this paper is mainly intended to deal with the angiospermous species, it may be mentioned incidentally that such new distributions have also come to light in respect of cryptogamic plants. To cite just one instance, *Andraea rupestris* Hedw which was previously known from Sikkim (based on a collection of J. D. Hooker) has been collected for the first time in western Himalaya in the Mana region of north Garhwal.

REFERENCES

CORRELL, D. S. 1950. *Native Orchids of North America*. Chronica Botanica Co., Waltham, Mass.

FOSTER, A. S. 1963. The morphology and relationships of *Circaeaster*. *J. Arnold Arbor.* **44:** 299-327.

HOLTTUM, R. E. 1957. *A Revised Flora of Malaya*. Vol. 1 *Orchids of Malaya*. Second edition, Singapore.

PENNELL, F. W. 1943. The Scrophulariaceae of the western Himalayas. *Acad. Nat. Sci. Philad. Monogr. No.* **5:** 66-67.

VAN STEENIS, C. G. G. J. 1934. On the Origin of Malaysian Mountain Flora, pts. 1-2 *Bull. Jard. bot. Buitenz.* ser. III, **13:** 135-262; 289-417.

Adv. Pl. Morph. 1972: 137-152

MORPHO-TAXONOMIC STUDIES ON INDIAN GUTTIFERAE. THE GENERA *MAMMEA* LINN. *S. l.* AND *KAYEA* WALL.

By J. K. Maheshwari

National Botanic Gardens, Lucknow-1, India

Abstract

This study deals with morphologic-taxonomic observations on the guttiferous genera *Mammea* (including *Ochrocarpos*) and *Kayea* as occurring in India. The features of botanical interest in the family, especially in the tribe Calophylleae (*Calophyllum, Kayea, Mesua, Poeciloneuron, Ochrocarpos, Mammea*) are mentioned. The *Taeniopteris* type of leaves showing a distinct midrib and parallel lateral veins running direct to the leaf margin occurs in the genera *Calophyllum, Pentadesma, Symphonia, Mammea, Monobea, Platonia*, and shows advanced lines of evolution. The *Mammea-Ochrocarpos* alliance and the reduction of the genus *Kayea* to *Mesua s. ampl.* are discussed. Three species of the inclusive genus *Mammea s. ampl.* are recognized in the area, including the mamey or mammee-apple (*M. americana* Linn.) introduced from tropical America. The genus *Kayea*, represented by three species in India, is treated under the amplified genus, *Mesua*. A key to the species is provided, together with notes on their systematics, types and distribution. The species, *Ochrocarpos siamensis* Anders. of Indian floras is referred to *Mammea nervosa* (Kurz) Kosterm. The problems of ill-defined generic-pairs and segregates, namely *Mammea-Ochrocarpos, Mesua-Kayea, Garcinia-Xanthochymus, Garcinia-Rheedia, Hypericum-Ascyrum, Hypericum-Sanidophyllum*, etc. and the reduction or segregation of allied guttiferous taxa are highlighted.

Introduction

The Guttiferae (nom. alt. Clusiaceae) represents one of the largest pantropical and megatherm families which is richly developed in the tropics. It embraces ca 40 genera and 1000 species distributed in tropical Asia from India to Malesia, Queensland, New Caledonia, tropical America, Africa, and Madagascar. Amongst the families which have a circumtropical range, the Guttiferae together with the Theaceae (ca 600 species) and Dipterocarpaceae (580 species) form a naturally related tropical group. The fossil woods (e. g., *Kayeoxylon assamicum, Guttiferoxylon indicum, Mesuoxylon arcotense, Calophylloxylon indicum*) and fossil leaves resembling those of the modern genera *Kayea, Mesua, Calophyllum*, etc., have been recorded from the Tertiary beds of Assam, South India and Rajasthan. Some of the forms were widespread and have now become limited in distribution (Prakash, 1965). The genus *Poeciloneuron* (2 species) is endemic in South India, while *Garcinia* (435 species), *Calophyllum* (188 species; also neotropical), and *Mesua*, including *Kayea* (41 species) are found from tropical Africa to Malesia and Queensland. The genus *Mammea*, including *Ochrocarpos* (49 species) has a wide distribution in tropical America, Africa, Asia, Malesia, Malaysia, and Madagascar.

The family derives its name from *gutta* or gamboge, a yellow resinous juice that can be tapped from the bark of Siamese Gamboge Tree (*Garcinia hanburyi*) and allied species. Many modern taxonomists use the alternate familiar name, Clusiaceae derived from the name of the type genus, *Clusia* Linn. which is distributed in tropical and subtropical America. In recent systems of classification (Hutchinson, 1959, 1969; Takhtajan, 1959), the subfamily Hypericoideae has been raised to the familial level, namely Hypericaceae. However, if this family is united with Guttiferae, the correct name under Article 57 of the Edinburgh Code, 1964 (ICBN, ed. Lanjouw et al., 1966) is Guttiferae (nom. alt. Clusiaceae). The chief characteristic features of the family are: simple, opposite (alternate in Kielmeyeroideae), exstipulate, penninerved leaves, often with resin ducts and secretory cavities in the shape of pellucid dots or canals: absence of bud scales; actinomorphic, hermaphrodite or unisexual flowers with imbricate or decussate sepals; stamens as a rule indefinite, free or united at base in bundles or in a tube; disc often present: ovary uni- to multilocular with 1-many ovules per locule; placentation axile or basal, rarely parietal (e.g., *Allanblackia*); seeds often arillate, without endosperm. The parallel venation of leaves, common in monocotyledons, has been found characteristic of a number of genera in the family, e.g., *Calophyllum*, *Pentadesma*, *Symphonia*, and in *Endodesmia* (Hypericaceae). The plants belonging to the form genus *Taeniopteris* show a distinct midrib from which parallel lateral veins run direct to the leaf margin. This form occurs in ferns, pteridosperms, cycads, Glossopteridae and angiosperms. Among angiosperms, the *Taeniopteris* type occurs in *Calophyllum* in the Guttiferae, where there is no reticulum and a marginal nerve is the only advance over the fossil forms. The vein pattern in *C. inophyllum* lacks in diversity and suggests its primitiveness (Kundu and Gupta, 1967). Other genera of Guttiferae (e.g., *Mammea*, *Monobea*, *Platonia*) have taeniopteroid leaves with the intercostal areas filled with a minor reticulum of intermediate to advanced angiosperm type suggesting the line of evolution in this family (Melville, 1969).

The family is quite rich in economic plants which have manifold uses on account of their often hard and durable wood (e. g., *Mesua ferrea*, Ceylon Ironwood, Ironwood of Assam; *M. assamica*, Sia-nahor; *M. floribunda*; *Calophyllum inophyllum*; Alexandrian Laurel; *C. elatum*, Poonspar Tree; *Poeciloneuron indicum*, Ballagi), resin ducts containing gum-resin and oleoresin (especially in the genera *Calophyllum*, *Clusia*, and *Garcinia*), succulent or drupaceous and often delicious fruits (e. g., *Garcinia*, *Rheedia*, *Pentadesma*) and fat-rich seeds (e. g., *Allanblackia floribunda*, Tallow Tree; *Pentadesma butyracea*, Butter Tree; *Garcinia indica*, Kokam Butter Tree).

The Guttiferae of India has been studied by Choisy (1851), Planchon and Triana (1860-1862, 1861), Anderson (1874), Vesque (1893), Maheshwari (1960, 1963, 1964) and others. Our knowledge of the family on world-basis has been summarized by Engler and Keller (1925). In Engler's *Syllabus der Pflanzenfamilien*, Melchior (1964) treated this family as composed of 6 subfamilies including the Hypericoideae and 9 tribes:

I. Subfam. Kielmeyeroideae:
   (i) Tribe Kielmeyereae;
   (ii) Tribe Caraipeae.
II. Subfam. Calophylloideae:
   (i) Tribe Calophylleae;
   (ii) Tribe Endodesmieae.

III. Subfam. Clusioideae:
  (i) Tribe Clusieae;
  (ii) Tribe Garcinieae.
IV. Subfam. Moronoboideae.
V. Subfam. Lorostemonoideae.
VI. Subfam. Hypericoideae:
  (i) Tribe Vismieae;
  (ii) Tribe Cratoxyleae;
  (iii) Tribe Hypericeae.

Some recent authors, including Lawrence (1951), Hutchinson (1959) and Takhtajan (1959) have split the group into two families, elevating Engler's Hypericoideae to the familial level. It has, however, been suggested that a re-evaluation of the claim of the Hypericaceae to familial rank is necessary because of the close inter-relationship within these groups. The Hypericoideae seems to be related to the Calophylloideae and to the Clusioideae through *Hypericum* (Vestal, 1937; Lawrence, 1951; Adams, 1962). Hutchinson (1969) opines that the amalgamation of the Hypericaceae and Guttiferae results in a cumbersome group of considerable size. He divides the family into following 5 tribes, with two major changes over the Bentham-Hooker classification (1862)—the inclusion of a new tribe Allanblackieae in the Guttiferae and the upgradation of the Quiineae to the familial level, Quiinaceae:

Tribe 1. Moronobeae;
Tribe 2. Calophylleae;
Tribe 3. Clusieae;
Tribe 4. Garcinieae:
Tribe 5. Allanblackieae.

The genera under study, namely *Mammea s. ampl.* (including *Ochrocarpos*) and *Kayea* belong to the tribe Calophylleae which also includes the genera *Calophyllum*, *Mesua*, and *Poeciloneuron*. The tribe Calophylleae is characterised by polygamous or hermaphrodite flowers, stamens free or united only at the base, style distinct, fruit rarely dehiscent (e.g., *Mesua*) and embryo consisting of thick, fleshy cotyledons and very short radicle. Tixier (quoted in Darlington and Wylie, 1955) examined the chromosomes of *Calophyllum inophyllum*, *Mesua ferrea*, and *Ochrocarpos siamensis*, and found uniformity in the chromosome number of the three genera, with $2n=32$. Recent studies suggest the reduction of *Ochrocarpos* to *Mammea*, and of *Kayea* to *Mesua* (De Wilde, quoted in Kostermans, 1961; Kostermans, 1969). The common and differential features of the various genera of the tribe Calophylleae are summarized in Table I. The present work deals with a morphologic taxonomic study of Indian species of *Mammea s. ampl.* (including *Ochrocarpos*) and *Kayea*, both of which are in need of intensive botanical and systematic investigations.

The herbarium specimens cited in the present study are housed in the Central National Herbarium, Sibpur, near Calcutta (CAL*), Herbarium of National Botanic Gardens, Lucknow (LWG), Blatter Herbarium, St. Xavier's College, Bombay (BLAT), and the Regional Herbarium of the Botanical Survey of India at Poona (BSI), Shillong (ASSAM) and Coimbatore (MH). Living plants of *Mammea suriga* were examined in the Indian Botanic Garden, Sibpur, Howrah and the National Botanic Gardens, Lucknow.

THE *MAMMEA-OCHROCARPOS* ALLIANCE

The genera *Mammea* and *Ochrocarpos* are closely related in their vegetative and floral morphology. They have many

---

*The citation of various herbaria of the world in the text for purpose of the location of types and specimens, is abbreviated according to Lanjouw and Stafleu's *Index Herbariorum* Part I (ed. 5, 1964).

features in common, e.g., flowers polygamous, calyx closed before anthesis, later splitting into 2 (sometimes 3) valves or sepals, petals 4 (rarely more), stamens numerous, anthers dehiscing by a longitudinal slit, style short, ovules 4, seeds enclosed in pulp, etc. The genus *Mammea* was established by Linnaeus (Sp. Pl. 512. 1753 and Gen. Pl. ed. 5. 228. 1754). Of the two Linnaean species, *M. americana* and *M. asiatica,* the latter represents *Barringtonia asiatica* (Linn.) Kurz. The genus *Ochrocarpos* was founded in 1806 by du Petit-Thouars (Gen. nov. Madagascar 15. 1806). Bentham and Hooker (in Gen. Pl. 1: 176. 1862) distinguished between *Mammea* and *Ochrocarpos* on the basis of differences in cotyledons, in style and stigma, and the area of distribution. They included the genera *Ochrocarpos* and *Mammea* in the tribes Garcinieae and Calophylleae respectively. This distinction has been maintained by Engler and Keller (1925) who had, however, put *Mammea* and *Ochrocarpos* close together; the former characterized by ovary 4 to 2-locular with 4 ovules, flowers axillary and stigma 2 to 4-lobed; the latter by ovary 2-locular, with at least 2 seeds, flowers in clusters and peltate, entire stigma. In Afro-Asian floras, the Old World species are usually assigned to the genus *Ochrocarpos.* Recently, Kostermans (1961) came to the conclusion that the Old World species of *Ochrocarpos* should be referred to *Mammea.* De Wilde (quoted in Kostermans, 1961) cited many cases which show that there should be transitional taxa between *Ochrocarpos* and *Mammea.* He also demonstrated that *Ochrocarpos* could not be maintained as a separate genus;

TABLE I

MORPHOLOGICAL FEATURES OF THE GENERA OF CALOPHYLLEAE

Genus	Area of distribution	Sepals	Ovary	Ovules per ovary	Style	Stigma	Seeds per fruit
Calophyllum	Paleotropics; trop. America	2 or 4	unilocular	1	1	peltate	1
Kayea	Indo-Malesia to Queensland	4	unilocular	4	1	4-fid	1-4
Mesua	Indo-Malesia	4	bilocular (also unilocular in fruit)	4	1	peltate (sometimes incised)	1-4
Poeciloneuron	S. India (Malabar), endemic	4-5	bilocular	4	2	pointed	1-2
Ochrocarpos	Paleotropics	2, rarely 3	2(-3)-locular, later4(-6)-locular	4	1	peltate, 3-lobed	1-4
Mammea	Trop. America and W. Africa	2	4-(2)-locular	4	1	peltate, 2 to 4-lobed	1-4

the differential characters and correlation of characters being too inconstant or weak to be of taxonomical value. Of late years, a re-evaluation of the generic status of *Ochrocarpos* and its separation from *Mammea* has been advocated; the former distinguished by the bands of secretory canals of the leaves which cross the secondary nerves and the more or less fused filaments: the latter by areoles with a transparent gland in their centre and the free filaments. The Old World species are mostly constant in the above-mentioned characters (Kostermans, 1961). However, its exact limits can only be determined after a detailed study of Old World species found in Asia, Malaysia, Madagascar and the Pacific. The following species occur in India, including the mamey-apple (*M. americana* Linn.) which has been introduced from America.

Mammea Linn. [Sp. Pl. 512. 1753 and Gen. Pl. ed. 5. 288. 1754] emend. De Wilde in Acta Bot. Neerl. 5 (2): 172, 176. 1956 and in Belmontia, Taxon. fasc. 1: 172. 1957; Kostermans in Comm. For. Res. Inst. Indones. Bogor Nr. 72: 2. 1961.

Ochrocarpos du Petit-Thouars, Nov. Gen. Madagasc. 15. 1806; Anderson in Hook. f. Fl. Brit. India 1: 270. 1874: Gamble, Fl. Madras 1: 75. 1915; Ridley, Fl. Mal. Pen. 1: 180. 1922; Kanjilal *et al.*, Fl. Assam 1(1): 110. 1934.

Calysaccion Wight, Ill. Ind. Bot. 1:130. 1840 & Icon. 6: 14. t. 1999. 1853.

Potamocharis Rottb. in Act. Litt. Univ. Hafn. 1: 296. 1778.

Small or medium-sized, resiniferous trees. Bark usually smooth, yellowish outside, red inside, containing white or usually yellow sap or latex. Leaves opposite or subverticillate, usually coriaceous, smooth, glabrous, glossy dark green above, pinnately nerved, petiolate, exstipulate; venation dense and areolate, with a pellucid gland in the centre of each areole; hypsophylls (scales) present at the base of leaves. Flowers polygamous, dioecious or hermaphrodite, solitary or fascicled in reduced cymes, axillary or usually on tubercles of bare branches and/or trunk, pedicellate, surrounded at the base by a number of decussate bracts. Buds mucronulate. Calyx united in the bud, at anthesis splitting into two convex halves, more or less persistent in fruit. Petals 4 (rarely more), decussate, caducous, usually white. Stamens numerous, usually free; filaments slender, white; anthers oblong, dehiscent by a longitudinal slit. Ovary in male flowers absent or strongly reduced; in hermaphrodite flowers ovary sessile, bilocular with 2 ovules per locule or tetralocular with one ovule per locule (septa sometimes incomplete); style short, topped by a broad peltate, 2 to 4-lobed stigma. Fruits drupaceous, indehiscent, usually unilocular, sometimes plurilocular; pericarp leathery. Seeds surrounded by an usually edible, transparent pulp.

Type species.—M. americana Linn. Sp. Pl. 512. 1753.

Distribution.— Circumtropical—tropical Asia, Africa and America, Malaysia, Malesia, Madagascar, New Caledonia; 3 species recorded in India, including *M. americana* Linn. introduced from tropical America.

KEY TO THE SPECIES

A. Fruits multilocular, globose; leaves oblong-obovate, rounded or blunt at the apex, 7.5-12 cm. broad in the middle........... 1. *M. americana*

A. Fruits monolocular, ovoid; leaves elliptic to linear-oblong or oblong-lanceolate, obtuse to subacuminate, 4-7 cm. broad in the middle:

B. Lateral nerves closely spaced, 2-3 mm. distant; pedicels above 2.5 cm. in length; petals 6; fruits ovoid...... **2.** *M. nervosa*

B. Lateral nerves spaced ca 1 cm.; pedicels 1.5-2 cm. long; petals 4; fruits obliquely ovoid...... **3.** *M. suriga*

1. *Mammea americana* Linn. Sp. Pl. 512. 1753; Fawcett & Rendle, Fl. Jamaica 5: 201. 1926; Bailey, Stand. Cyclop. Hort. 2: 1975. 1929.—PLATE I, FIGS. 1-3.

Small or medium-sized, slow-growing trees, 12-20 m. high. Leaves broadly oblong-obovate or elliptic-obovate, 10-25 x 7.5-12 cm., glabrous, entire, rounded or blunt at the apex, base wedge-shaped, obtuse or rounded coriaceous, dark green, glossy on the upper surface, marked with numerous fine transverse reticulated veins and pellucid, gland-dots; petiole stout, 1 cm. or more in length. Flowers polygamous, solitary or fasciculate, white, fragrant, ca 2.5 cm. in diam. Sepals 2, oval-roundish, 1.3-1.7 cm. long. Petals usually 5, obovate, 1.7-2 cm. long. Anthers oblong, laterally dehiscent. Fruits 15-20 x 7.5-15 cm., oblate to globose, apiculate, indehiscent, reddish-green or russet-brown with thick leathery skin. Seeds 1-4, resinous, embedded in an orange-coloured, sweetish, aromatic pulp.

Type.—In Herb. LINN (type locality: Hispaniola, Jamaica).

Herbarium specimens examined.—BENGAL: Royal (now Indian) Botanic Garden, Sibpur, Calcutta, cult., in 1856, without collector's name, acc. no. 47235 (CAL). AMERICA: Flora Martin. no. 144, without collector's name acc. no. 47751 (CAL); *F. Urban* 1656, vide P. Sintenis, Plantae Portoricensis. acc. no. 47750 (CAL).

Distribution.—Native of West Indies; widely cultivated in many tropical countries for its edible fruit.

Vern. names.—Eng.: Mamey, Mammee-apple, St. Domingo Apricot, Mammee-American mammee tree.

2. *M. nervosa* (Kurz) Kostermans, Monogr. *Mammea* in Comm. For. Res. Inst. Indones. Bogor nr. 72: 25. f. 20. 1961—PLATE I, FIGS. 4-9.

Ochrocarpos nervosus Kurz, For. Fl. Brit. Burma 1: 94. 1877.

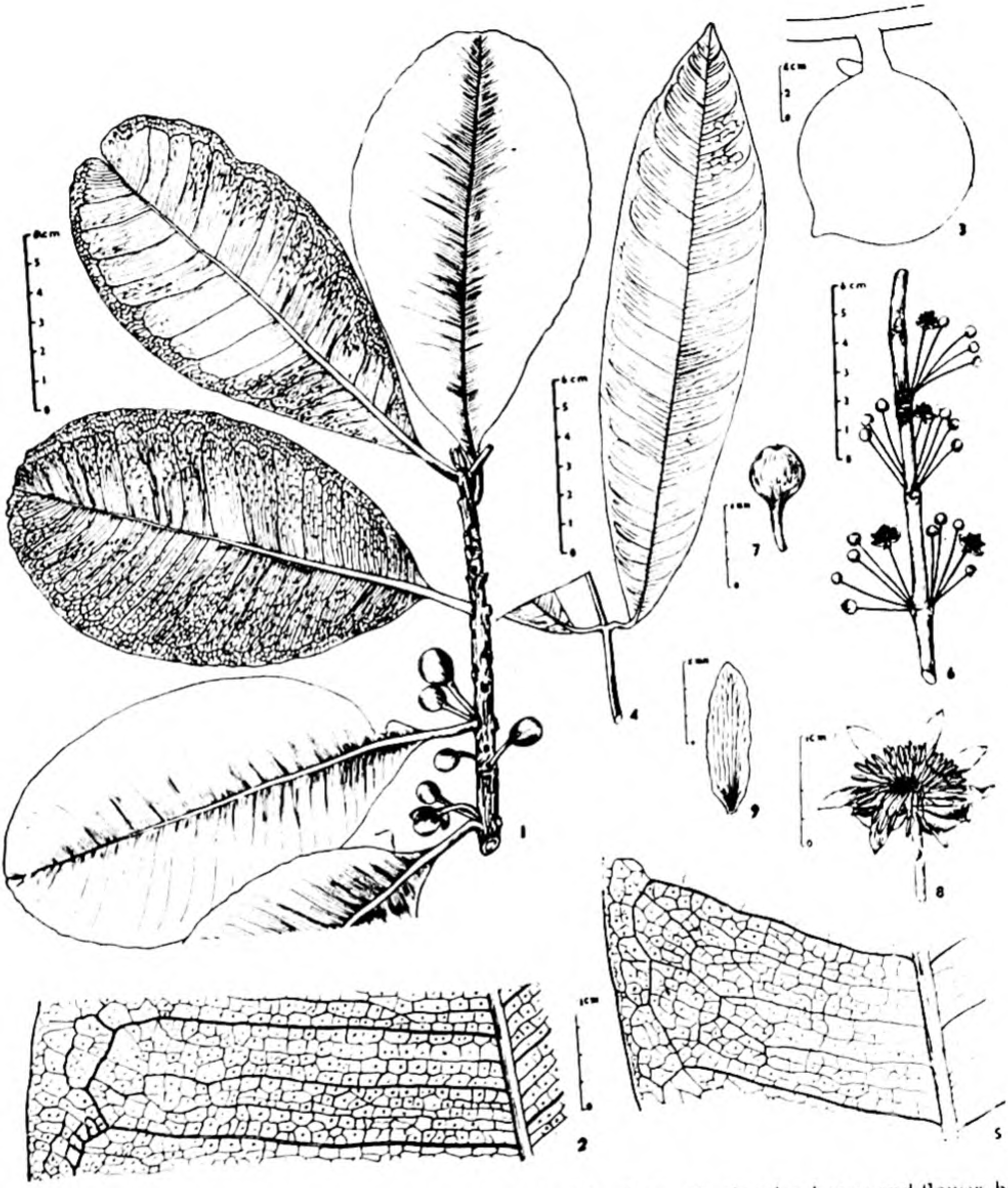
O. siamensis Anderson in Hook. f. Fl. Brit. India 1: 270. 1874, *pro parte;* Kanjilal *et al.*, Fl. Assam 1 (1) : 111. 1934, non *Mammea siamensis* (Miq.) Anderson in J. Linn. Soc. Bot. 9: 261. 1867.

Trees, up to 15 m. high. Bark greyish-brown, reddish inside, turning brown. Leaves rigidly coriaceous, linear-oblong to oblong or oblong-lanceolate, 12-24 x 4-5.5 cm., acute to subacuminate, base acute; lateral nerves numerous, closely spaced, anastomosing very closely to the margin; petiole stout, up to 1 cm. long; hypsophylls (scales) minute. Flowers solitary or in fascicles of 3-7, white, fragrant; pedicels slender, 2.5 - 5 cm. long. Buds globose. Sepals 2, ca 5 mm. long. Petals 6, obtuse or rounded, as long as or shorter than the sepals, obovate-oblong or broadly oblong-lanceolate. Stamens numerous; filaments filiform. Ovary globose, narrowed in a short, thick style; stigma 2-lobed. Fruits ovoid, mucronate, ca 3 cm. long, glabrous.

Flowers.—March. *Fruits* : May-July.

Type.—*Kurz* s. n. (K, P, CAL).

Herbarium specimens examined.—ASSAM: Khasia Hills, without collector's name, acc. nos. 47239 & 47240 (CAL); Tepaimukh, Lushai Hills, 150 m., *U. N. Kanjilal* 4767, Nov. 27, 1914 (CAL and ASSAM); Lushai Hills, *U. N. Kanjilal* 7642, March 24, 1921 (ASSAM). EAST BENGAL : Chittagong Hill Tracts, *J. L. Lister* s. n., in 1876 (CAL). BURMA: Arracan, *S. Kurz* s. n. (CAL).



FIGS. 1-9. Figs. 1-3. *Mammea americana.* Fig. 1., Tip of branch showing leaves and flower buds. Fig. 2. Portion of undersurface of leaf showing pellucid glands (from herbarium sheet no. 47751, CAL. Fig. 3. Outline sketch of fruit. Figs. 4-9. *M. nervosa.* Fig. 4. Leaf showing nervation. Fig. 5. Portion of undersurface of leaf. Fig. 6. Flowering twig. Fig. 7. Flower bud. Fig. 8. Open flower. Fig. 9. Petal (Figs. 4, 6-9, after *U. N. Kanjilal* 7642, ASSAM; Fig. 5, after *S. Kurz* s.n., CAL).

Distribution.—Assam, Burma.

Vern. names.—Ass. : Suklong.

Notes.—Anderson (loc. cit.) and Kanjilal (loc. cit.) included the species in *Ochrocarpos siamensis* Anderson; the latter is distributed in Siam, Indonesia and East Burma, and does not occur in India. Anderson (loc. cit.) cites Kurz' specimen which differs from *O. siamensis* in the shape and nervation of the leaves.

3. *M. Suriga* (Buch.-Ham. ex Roxb.) Kostermans, Monogr. *Mammea* in Comm. For. Res. Inst. Indones. Bogor nr. 72: 23. f. 19. 1961—PLATE II, FIGS. 10-17.

Calophyllum suriga Buch.-Ham. ex Roxb. Hort. Beng. 93. 1814, nom. nud. and Fl. Ind. ed. Carey 2: 608. 1832.

C. longifolium Wall. Cat. 4851. 1831, nom. nud.

Mammea longifolia (Wight, Pl. and Tr. in Ann. Sci. nat. sér. 4, 15: 240. 1861; Wealth of India 6: 252. 1962.

Calysaccion longifolium Wight, Ill. Ind. Bot. 1:130. 1840 & Icon. 6:14. t. 1999. 1853.

Ochrocarpos longifolius (Wight) Anderson in Hook. f. Fl. Brit. India 1: 270. 1874; Kanjilal *et al.*, Fl. Assam 1(1): 111. 1934.

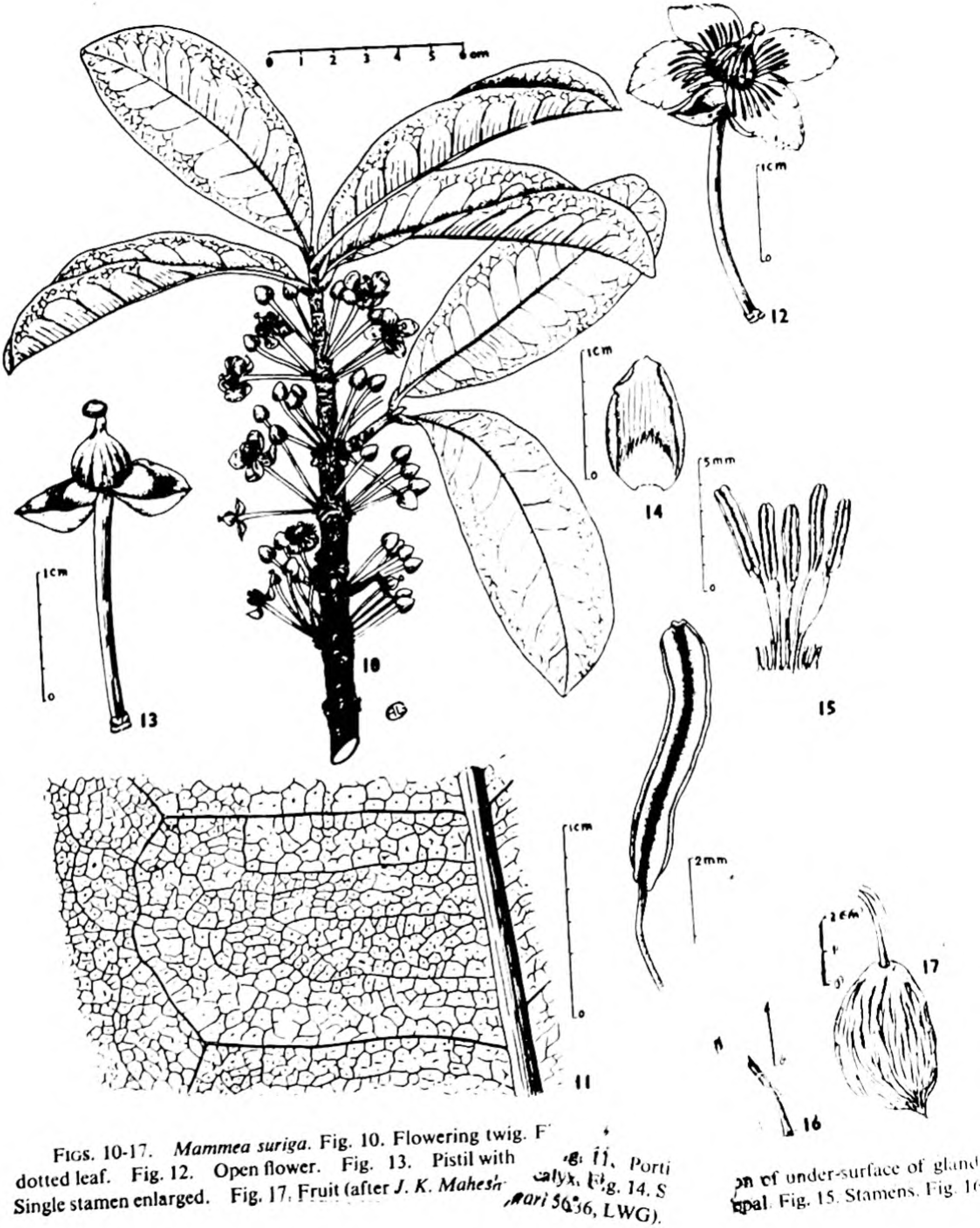
Trees, 12-18 m. high; young branchlets smooth, obscurely angled. Bark rough, peeling off in irregular rectangular pieces. Leaves thickly coriaceous, elliptical or oblong, 10-20 × 4-7 cm., obtuse or subacuminate, base obtuse; midrib stout, prominent; lateral nerves spaced ca 1 cm., faint, connected by a dense reticulation; marginal nerve faint at 1 cm. from margin; petiole stout, channelled above, 7-10 mm. long. Flowers ca 1 cm. in diam., white or pinkish, sweet-scented, often hermaphrodite in cultivation, axillary or in fascicles on nodes of old wood or in axils of fallen leaves; pedicles 1.5-2 cm. long. Buds globose, white streaked with red. Calyx-segments concave, reflexed during flowering. Petals 4, deciduous, oblong-obovate, acutish, up to 8 mm. long. Stamens ca 60. Ovary depressed-subglobose, 2 to 4-locular; style short, stout, subulate; stigma large, umbonate, peltate, obscurely sinuate or 2-lobed. Fruits obliquely ovoid, ca 2.5 cm. long, tipped by the hard, pointed style, stipitate, 1 to 4-seeded.

Flowers.–March-June. *Fruits.*–May-July.

Type: Roxburgh s. n. (BR); *Wallich* 4851 (K).

Herbarium specimens examined.—PENINSULAR INDIA: Madras, Herb. *Wight* 144(CAL); *Wallich* 4851, Herb. Madras (CAL); Northern Division, Madras, *Cleghorn* s. n. (CAL); Madras, cult., *J. S. Gamble* 17069, April 1886 (MH); Karwar-N. Kanara, *Bell* 5755, March 1919 (CAL); Katgal, Kanara, without collector's name, Feb. 1893 (CAL); North Kanara, *W. A. Talbot* 1816, May 1889 (BSI); Tungar Bassein, Thana dist., *G. M. Ryan* 846, May 16, 1903 (BSI); near Kanheri Caves, *R. K. Bhide* s. n., April 3, 1904 (BSI); Ganpati Pula, near Ratnagiri, *R. K. Bhide* s. n., May 24, 1904 (BSI); Khandala, *Bhiva* s. n., March 1891 (BSI); Victoria Gardens, Bombay, *R. R. Fernandez* R. 2413, July 10, 1956 (BLAT); Ankola, Bombay Pr., *J. C. Dhruna* s. n., Feb. 9, 1950 (CAL); without exact locality, *Gibson* s. n., acc. no. 47234 (CAL). BENGAL: Indian Botanic Garden, Calcutta, cult., without collector's name (CAL). ORISSA: W. Sonakalla, Khurdha, introduced, *J. S. Gamble* 9319 (CAL). UTTAR PRADESH: National Botanic Gardens, Lucknow, cult. *P. C. Kanjilal* 252, April 3, 1952; *D. D. Awasthi* 158 & *J. K. Maheshwari* 5636, April 7, 1967 (LWG).

Distribution.—Evergreen forests of Western India from Khandala to Malabar and Coimbatore, up to 600 m. alt. cultivated elsewhere for its handsome foliage


0 1 2 3 4 5 6 cm
1cm
0
12
1cm
0
14
5mm
0
15
1cm
0
13
10
1cm
0
11
2mm
2cm
1
0
16
17

FIGS. 10-17. *Mammea suriga*. Fig. 10. Flowering twig. Fig. 11. Portion of under-surface of gland dotted leaf. Fig. 12. Open flower. Fig. 13. Pistil with calyx. Fig. 14. Sepal. Fig. 15. Stamens. Fig. 16 Single stamen enlarged. Fig. 17. Fruit (after *J. K. Maheshwari* 5636, LWG).

and sweet-scented flowers.

Vern. names.—Bomb.: Suringi, Tambranagkesar, Surang; Konkan: Ran-undi; Mar.: Punnag, Suringi; Guj.: Rati-nagkesar; Tam.: Nagappu, Nagashop-pu, Nagesar-pu, Surabunnai; Tel.: Suraponna. Kan.: Wundi, Laringi (male), Pune (female), Suringi, Gardundi; Mal.: Seraya, Suram-punna; Or.: Churiana; Sans.: Naga-kesaram-pushpam.

REDUCTION OF THE GENUS *KAYEA* WALLICH TO *MESUA* LINN.

The genus *Kayea* was established by Wallich in *Plantae Asiaticae Rariores* (1832) for a large tree (*K. floribunda*) found in the tropical forests of Eastern Himalayas and hills of Assam and East Bengal. The genus belongs to the tribe Calophylleae and approaches both in habit and generic characters to the genus *Mesua* which is also widely distributed in Indo-Malesia. Bentham and Hooker (in Gen. Pl. 1: 176. 1862) and Anderson (in Hook. f. Fl. Brit. India 1: 259. 1874) placed the genera *Kayea* and *Mesua* close together; the former has an unilocular ovary with 4 ovules and one seed, single style and a 4-fid style-apex, wheras the latter has a bilocular ovary with 4 ovules, single style and a peltate stigma. Anderson (loc. cit.) described the species, *M. lepidota* which, according to him, probably represents a new genus between *Kayea* and *Mesua* on account of the occurrence of small, lignified, non-accrescent sepals. Recently, Kostermans (1969) examined living collections from trees of *M. ferrea* growing in the Bogor Botanic Garden, Indonesia and found both one-celled and 2-celled fruits with 1 or 2 seeds on the same tree. In some species of *Kayea*, 2- and 4-seeded fruits were also observed, e.g., *K. floribunda* (Kanjilal, 1934). Besides, there have been noted all gradations between the peltate stigma of *Mesua ferrea* (which is sometimes incised) and the 4-fid style-apex of *Kayea*. The genus *Kayea* has, therefore, been merged under *Mesua*. The inclusive genus *Mesua* comprises more than 40 species distributed in tropical Asia and Indo-Malesia. A detailed study of the genus *Mesua s. str.* in India has been made by the author (Maheshwari, 1963). The following species of *Kayea* occur in India (Wallich, 1832; Brühl and King, 1896; Prain, 1901) and are treated under the inclusive genus *Mesua s. ampl.*

Mesua. Linn. [Sp. Pl. 515. 1753] emend. Kostermans in Reinwardtia 7: 425. 1969; Maheshwari in Bull. bot. Surv. India 5: 335. 1963.

Kayea Wall. Pl. As. Rar. 3: 4. t. 210. 1832; Anderson in Hook. f. Fl. Brit. India 1: 276. 1874; Vesque in DC. Mon. Phan. 8: 610. 1893; Kanjilal et al., Fl. Assam 1(1) : 112. 1934.

Vidalia F.—Villar in Blanco, Fl. Filip. Noviss. Append. 17. 1880.

Shrubs or trees. Leaves opposite, polymorphic, entire, pellucid-dotted, petiolate. Flowers solitary, fasciculate or in panicles, variable in size, hermaphrodite. Sepals and petals 4 each, imbricate; sepals usually concrescent with fruit. Stamens numerous, hypogynous; filaments filiform, free or connate at the base; anthers 2-celled, variously shaped, dehiscing at the top or by lateral vertical clefts. Ovary uni- or bilocular, also incompletely bilocular; ovules 4, erect; style 1; stigma peltate, incised or 4-fid. Fruits fleshy, drupaceous or capsular nuts, dehiscent or indehiscent, 1 to 2-celled, subtended or encased by lignified, accrescent sepals. Seeds 1-4, exarillate, without endosperm; cotyledons thick, free.

Type species.—*M. ferrea* Linn. Sp. Pl. 515. 1753.

Distribution.—South-East Asia, from

Indo-Malesia to Queensland.

KEY TO THE SPECIES

A. Flowers ca 8 mm. in diam., on short panicle branches in 8-15 cm. long panicles; leaves ovate to elliptic-lanceolate............**1.** *M. assamica*

A. Flowers 1.2-2.5 cm. in diam., in clustered racemes or lax panicles; leaves oblong to lanceolate:

B. Petiole ca 7 mm. long; flowers in fasciculate, 3.5-6.5 cm. long racemes crowded on the tips of branchlets; ovary boldly ridged.......**2.** *M. manii*

B. Petiole 1.5 -2.5 cm. long; flowers in terminal, 15 cm. or more long panicles; ovary not ridged. .....**3.** *M. floribunda*

1. *Mesua assamica* (King and Prain) Kostermans in Reinwardtia 7: 426. 1969—PLATE III, FIGS. 18-28.

Kayea assamica King and Prain in Indian For. 27: 62. 1901; Kanjilal et al., Fl. Assam 1: 113. 1934; Wealth of India 5: 316. 1959.

Tall, evergreen trees, up to 25 m. high with a straight bole. Branches greenish-yellow. Bark grey. Leaves ovate or elliptic-lanceolate, 10-16 × 4-5 cm.. shortly acuminate, cuneate at base, somewhat shining above, dull beneath; lateral nerves 15-30, arched, not prominent on either surface, forming an uneven marginal vein; petiole slender, ca 2 cm. long. Flowers white, ca 38 mm. across, in axillary or terminal, fascicled, 8-15 cm. long panicles with short glabrous bracteate branches; pedicels in flower very slender, much enlarged and thickened in fruit; bracts and bracteoles opposite, small, caducous. Buds globular. Sepals 4, in 2 pairs, imbricate; outer pair orbicular, ca 5 mm. long, much enlarged in fruit; inner pair spathulate. Petals 4, ca 4 mm. long, suborbicular, shorter than sepals. Stamens numerous, longer than sepals; filaments free, capillary; anthers globose. Fruits depressed-globose, ca 2.5x 4.5 cm., corky outside, almost entirely enveloped by the hard, accrescent sepals; latex yellowish-brown. Seeds usually solitary reddish-brown, smooth.

Flowers.—April-July. *Fruits.*—Feb-April.

Type.—*Barker* s. n.; *Young* s. n., North Lakhimpur, Assam (BM, G, K, CAL).

Herbarium specimens examined.—ASSAM: Dibrugarh, *H. G. Young* s. n., Dec. 1899 & June 29, 1900 (CAL): North Lakhimpur dist., Upper Assam, *Barker* s. n. (CAL): Dulong Reserve, Lakhimpur dist., 95 m., *U. N. Kanjilal* 3678, March 24, 1914 (ASSAM): Dirju, Lakhimpur dist., 90 m., *U. N. Kanjilal* 4398, April 6, 1914 (ASSAM): Lakhimpur dist., *P. C. Kanjilal* 10256, July 18, 1932 (ASSAM); Dulong R. F., *G. Panigrahi* 27688, March 9, 1962 (ASSAM).

Distribution.—Common in submontane forests of North Lakhimpur, Assam.

Vern. names.—Ass. : Sia-nahor.

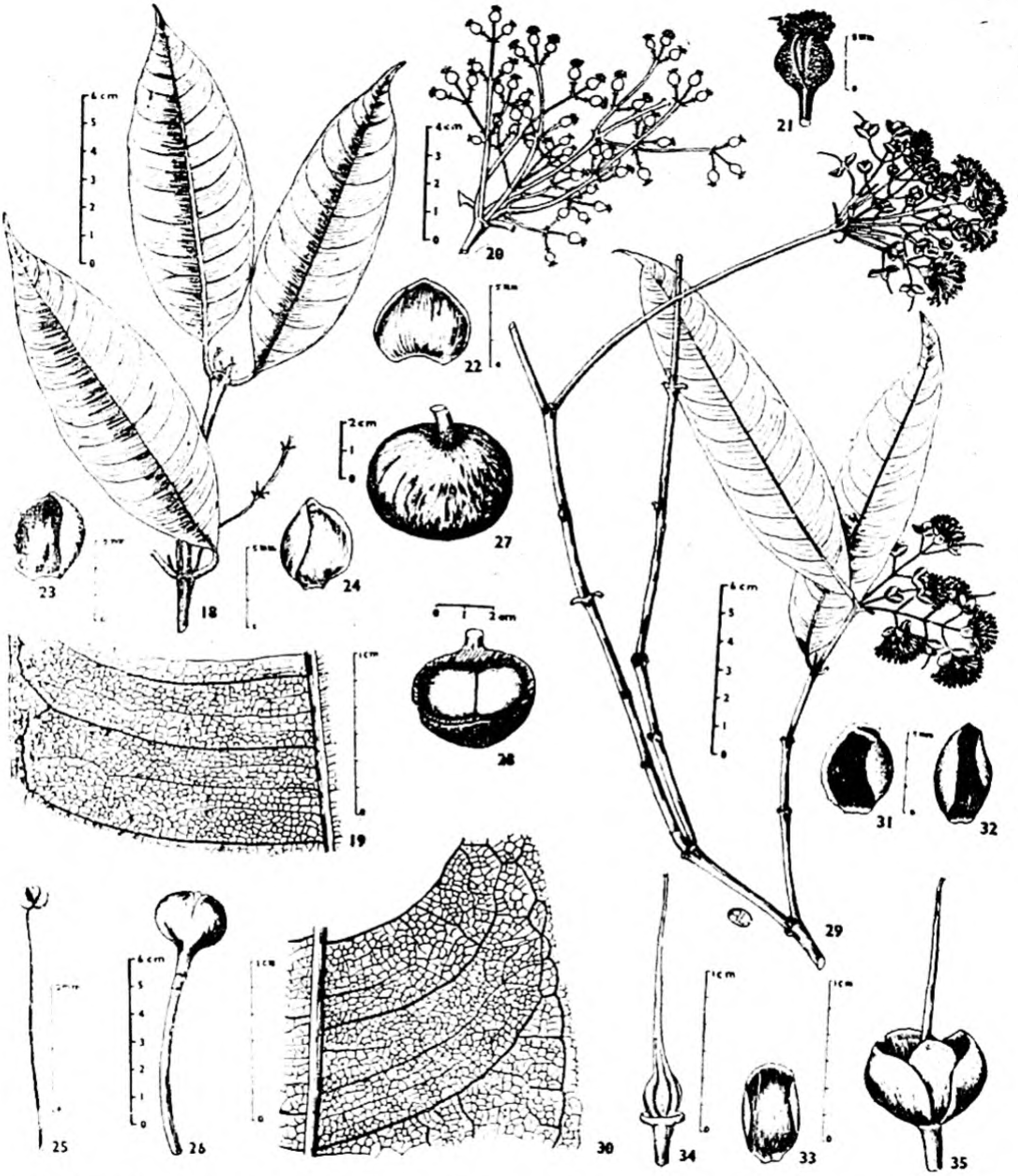
Notes.—The species closely allied to is *Mesua floribunda;* the latter distinguished by its much larger leaves with fewer, conspicuously arcuate, lateral nerves, copious racemes with larger flowers and its larger fruits.

2. *M. manii* (King) Kostermans in Reinwardtia 7: 428. 1969—PLATE III, FIGS. 29-35.

Kayea manii King in Ann. Roy. bot. Gard. Calcutta 5: 144. t. 174 A. 1896.

Mesua singaporeana Wall. Cat. 4836. 1831, nom. nud.

Glabrous trees. Young branches slender, polished. Bark pale brown. Leaves coriaceous, narrowly oblong, 12-17 × 3-4 cm., tapering at ends, apex acuminate, base acute; main lateral


6 cm
5
4
3
2
1
0
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
2 cm
1 cm
5 mm
0 1 2 cm

FIGS. 18-35. *Mesua assamica*. Fig. 18. Twig with leaves showing nervation. Fig. 19. Portion of under-surface of leaf. Fig. 20. Inflorescence. Fig. 21. Partially open flower. Fig. 22. Outer Sepal. Figs. 23, 24. Inner sepals. Fig. 25. Stamen. Fig. 26. Young fruit. Fig. 27. Mature fruit. Fig. 28. Cross-section of fruit Figs. 29-35. *M. manii*. Fig. 29. Flowering twig. Fig. 30. Portion of under-surface of leaf. Figs. 31-33. Sepals. Fig. 34. Pistil. Fig. 35. Young fruit with accrescent sepals (after *King's collector* s. n., CAL). (Figs. 18-19, 27, after *U. N. Kanjilal* 4398, ASSAM; Figs. 20-25, after *P. C. Kanjilal* 10256, ASSAM; Fig. 26, after *H. G. Young* s. n., CAL; Fig. 28, after *G. Panigrahi* 27688, ASSAM).

nerves 15-20 pairs, forming an undulated marginal nerve, intermediate nerves running about half way up, curving, somewhat prominent on the lower surface; petiole ca 7 mm. long. Flowers ca 12 mm. in diam., borne in several, fasciculate, 3.5-6.5 cm. long, slender racemes from axils; pedicels 1-1.5 cm. long. Sepals ovate-orbicular, concave. Petals broadly ovate, concave, slightly smaller than the sepals. Stamens much longer than the petals, numerous; anthers broadly ovate. Pistil longer than the stamens. Ovary conical, subcompressed, boldly ridged, glabrous, tapering into the cylindrical style; stigma with linear lobes. Fruits compressed, with persistent style, enveloped in the accrescent sepals.

Flowers.—January.

Type.—*E. H. Man*, Andaman Isl.; paratype: *Wallich* 4836 (CAL).

Herbarium specimens examined.—North Bay-Hill jungle. S. Andamans, *King's collector* s. n., Jan. 9, 1892 (CAL); Singapore, *Wallich* 4836 (CAL).

Distribution.—Andaman Islands, Singapore.

Notes.—The type specimen of this taxon (*E. H. Man* s. n.) matches perfectly with Wallichian specimen no. 4836 collected from Singapore in 1822, but is different from the specimen, *Maingay* (Kew distrib no. 177) collected from Malacca and the Wallichian specimen (in Herb. DC, P), on which Planchon and Triana based their species, namely *Kayea racemosa*.

3. *M. Floribunda* (Wall.) Kostermans in Reinwardtia 7: 427. 1969—PLATE IV, FIGS. 36-52.

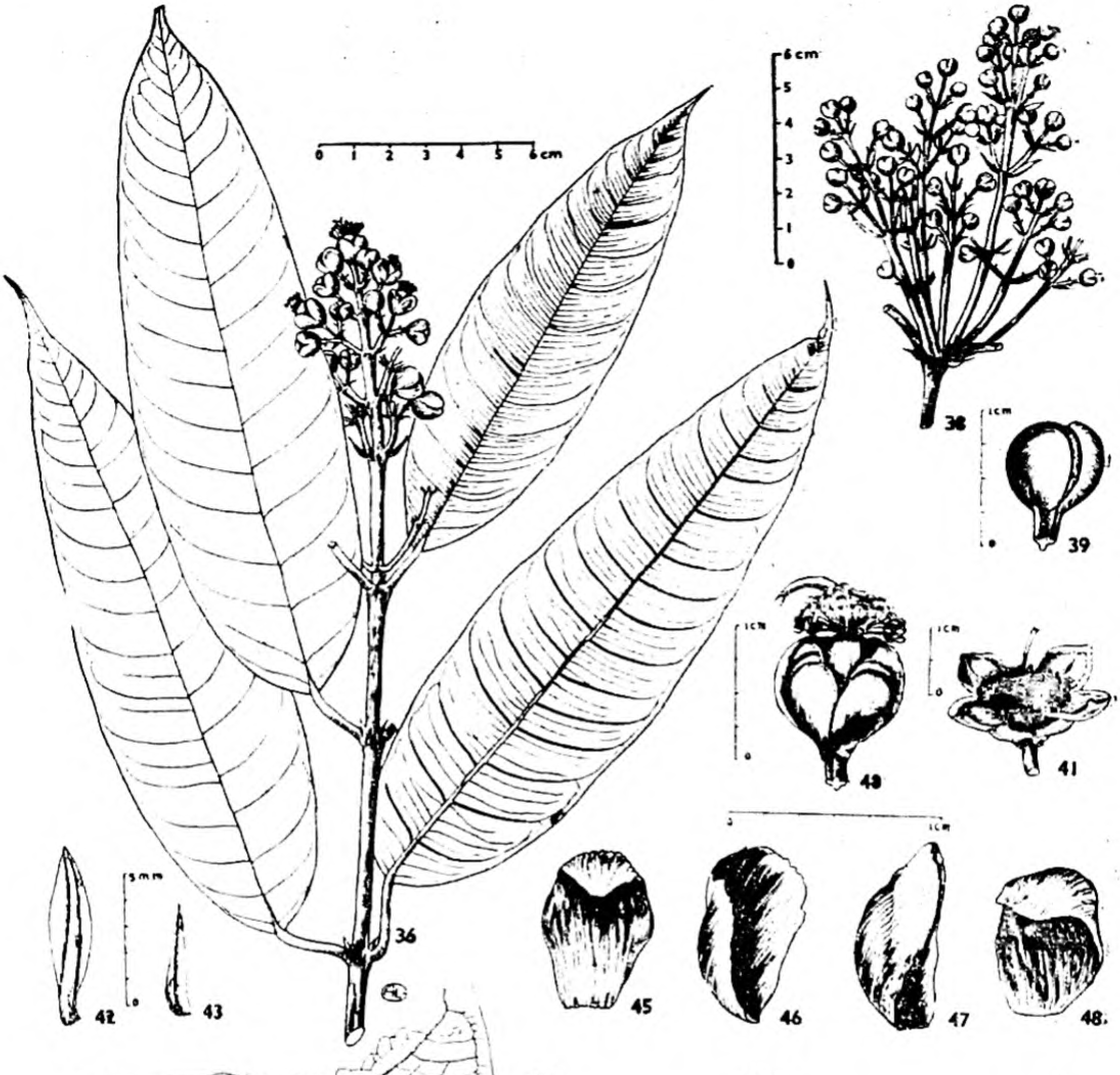
Kayea flouribunda Wall. Pl. As. Rar. 3:5. t. 210. 1832; Anderson in Hook. f. Fl. Brit. India 1: 276. 1874; *Kanjilal* et al., Fl. Assam 1: 113. 1934; Wealth of India 5: 317. 1959.

Tall, evergreen trees. Bark greenish-grey or brown, reddish inside, exfoliating in round or squarish flakes. Leaves narrowly or broadly oblong to lanceolate, 12-18 × 4-7 cm., acute or acuminate, base acute, cuneate or rounded, coriaceous, glabrous; lateral veins arched, meeting near the margin, prominent on the undersurface; petiole 1.5-2.5 cm. long. Flowers hermaphrodite, white with rosy edges, ca 2.5 cm. in diam., in terminal, many-flowered panicles; ultimate branchlets usually in 3-flowered cymes: branches and pedicels with two opposite bracts or bracteoles at their bases: pedicels 6-8 mm. long. Buds globose. Sepals 4, orbiculate, broader than long, accrescent in fruit. Petals 4, oblong-obovate or obovate. Stamens many; filaments filiform; anther-cells 2, reniform, golden-yellow. Ovary ovoid-conical. Fruits depressed-globose or transversely ellipsoid, ca 3.5 cm. in diam., indehiscent, resiniferous, covered by the accresecent, rugose, yellow sepals, 1 to 2-seeded. Seeds reddish-brown, smooth.

Flowers.—March-July. *Fruits.*—May-August.

Type.—*Wallich* 4840, Sylhet (BO, BR, G, K, LE, NY, US).

Herbarium specimens examined.—ASSAM · Cachar, without collector's name, acc. no. 47559 (CAL); Cachar, *G. Mann* s. n. May 1885 (CAL); Lungti, Lushai, *J. C. Prazer* s. n., July 9, 1889 (CAL); Cachar, Herb. *S. Kurz* 141 (CAL); Khasia, *G. Mann* s. n. (CAL); *Narundar, Pungi*, Cachar, *G. Mann* s. n., May 1885 (CAL); Khasia Hills, without collector's name, May 1850, acc. no. 47568 (CAL); Khasia, *J. D. Hooker* and *T. Thomson*, 300-900m. (CAL); without exact locality, *Griffith* s. n., acc. on. 47567 (CAL); Pangtum forests, 40 miles Shillong to


0 1 2 3 4 5 6 cm
6 cm
5
4
3
2
1
0
36
37
38
1 cm
39
40
41
5 mm
42
43
44
45
46
47
48
49
50
51
52
2 mm
3 mm
5 mm
1 cm

FIGS. 36-52. *Mesua floribunda*. Fig. 36. Flowering twig. Fig. 37. Portion of under-surface of leaf. Fig. 38. Inflorescence. Fig. 39. Flower bud. Fig. 40. Partially open flower. Fig. 41. Open flower. Figs, 42-43. Bracts. Fig. 44. Sepal. Figs. 45-48. Petals. Fig. 49. Stamen. Fig. 50. Style with 4-fid stigma. Fig. 51. Fruit. Fig. 52. Cotyledons (Figs. 36-37, 39-40, 44-52, after *P. C. Kanjilal* 10119, ASSAM; Figs. 38, 42-43, after *P. C. Kanjilal* and *Sri Ram* 10082, ASSAM; Fig. 41, after Wallich, Plantae Asiaticae Rariores 3: t. 210. 1832).

Dawki Road, K. and J. Hills dist., *S. R. Sharma* 12366, Aug. 28, 1935 (ASSAM); Chattak, Sylhet dist., *G. K. Deka* 20444, Feb. 8, 1941 (ASSAM); Baghmara, Garo Hills dist., *P. C. Kanjilal* 10119, May 11, 1932 (ASSAM); Mahadeo forests, K. and J. Hills. *P. C. Kanjilal and Sri Ram* 10082, May 17, 1932 (ASSAM). EAST BENGAL: Sylhet, *Wallich* 4840 (CAL); Tillagarh Reserve, Sylhet. *A. Das* 10436, Nov. 14, 1932 (ASSAM); Koral, Pathania forest, Sylhet, *T. K. Gupta* s. n., July 1887 (BSI).

Distribution.—Eastern Himalayas, Sikkim, Bhutan and lower hill forests of Assam and East Bengal, up to an altitude of 100 m.

Vern. names.-Ass.-Bolong, Phai-hershei, Serpai, Darchong-khub, Karol, Kasukorol, Kurrum-jowa, Kurull.

DISCUSSION AND CONCLUSIONS

Recent studies on the morphology and taxonomy of Old World Guttiferae indicate that the delimitation and circumscription of a number of genera needs critical evaluation. Thus, the genera *Xanthochymus, Ochrocarpos* and *Kayea* have been sunk by taxonomists when intermediate species and transitional taxa were found to blur the supposedly natural generic boundaries. The following examples of ill-defined generic pairs are noteworthy in the Guttiferae and Hypericaceae: *Garcinia-Xanthochymus, Mammea-Ochrocarpos, Kayea-Mesua, Cratoxylum-Eliaea,, Hypericum-Ascyrum,* and *Hypericum-Sanidophyllum* (Adams and Robson, 1961; Kostermans, 1961, 1969; Maheshwari, 1964; Baas, 1970). The genus *Rheedia* from Madagascar and tropical Africa is not satisfactorily separable from *Garcinia* and, according to Robson (1961), should probably be merged with it in any future monographic treatment. Such cases have arisen frequently when genus-forming name was available to Linnaeus from the earlier pre-Linnaean botanical literature and was, in many cases, mono- or ditypic. After the Linnaean definition of the genus, there followed an enormous influx of new species during the last two centuries. In recent years, a large number of new taxa belonging to *Garcinia, Mammea, Kayea,* etc., have been discovered in Papuasia, tropical Africa, Madagascar, New Guinea, Indonesia, Borneo, Malaysia, etc. and many more are to be found in other parts of the Old and New World tropics. There would thus be needed a thorough revision of the genera for Asia, Malaysia, Africa and Madagascar where the maximum number of species concerned is to be found. In many cases, the revision and monography of genera has proved difficult owing to lack of adequate material of all species and also field characters, especially in the case of plants exhibiting polymorphism (e.g., *Mesua ferrea*), dioecism and polygamo-dioecism (e.g., *Garcinia, Rheedia, Mammea, Clusia*). This is quite apparent from the recent works of Henderson and Wyatt-Smith (1956), Maheshwari (1960, 1963, 1964), Kostermans (1961) and Ewan (1962). Bass (1970) recognized the importance of floral and vegetative anatomy in defining the generic limits of closely allied guttiferous genera like *Eliaea* from Madagascar and *Cratoxylum* from Indo-Malesia. With the aid of important new data, either of a traditional morphological kind or those derived from experimental studies, it would, therefore, be pertinent to gather fresh evidences in support of the reduction or segregation of allied guttiferous taxa.

REFERENCES

Adams, P. 1962. Studies in the Guttiferae II. Taxonomic and distributional observations on North American Taxa. *Rhodora* **64:** 231-242.

—, and N. K. B. Robson. 1961. A re-evaluation of the generic status of *Ascyrum* and *Crookea* (Guttiferae). *Rhodra.* **63:** 10-16.

Anderson, T. 1874. Guttiferae. In Hooker f. *Flora of British India*. London. **1**:271-276.

Baas, P. 1970. Anatomical contributions to plant taxonomy I. Floral and vegetative anatomy of *Eliaea* from Madagascar and *Cratoxylum* from Indo-Malesia (Guttiferae). *Blumea* **18:** 369-391.

Bentham, G., and J. D. Hooker. 1862. *Genera Plantarum.* Vol. 1. London.

Brühl. P., and G. King. 1896. A century of new and rare Indian plants. *Ann. R. bot. Gdn.* (*Calcutta*) **5:** 144. t. 174A.

Choisy, J. D. 1851. Description des Guttiferes de l'Inde. *Mėm. Soc. Phys. Genève.* **12:** 381-440. tt. 1-4.

Darlington, C. D., and A. P. Wylie 1955. *Chromosome Atlas of Flowering Plants.* (Second edition). London.

Engler, A., and R. keller. 1925. Guttiferae. In Engler and Prantl, *Die Natürlichen Pflanzenfamilien* (Second edition). Leipzig. **21:** 154-237.

Ewan. J. 1962. Synopsis of the South American species of *Vismia* (Guttiferae). *Contr. U. S. natl. Herb.* **35:** 293-377.

Henderson, M. R., and J. Wyatt-Smith. 1956. *Calophyllum* Linn. *Gdns Bull. Straits Settl.* **15:** 285-375.

Hutchinson, J. 1959. *The Families of Flowering Plants. Dicotyledons.* (Second edition). Vol. I. Oxford.

——. 1969. *Evolution and Phylogeny of Flowering Plants. Dicotyledons: Facts and Theory.* London and New York.

Kanjilal, U. N., P. C. Kanjilal, and A . Das 1934. *Flora of Assam.* Vol. I Part 1. Calcutta.

Kostermans, A. J. G. H. 1961. A Monograph of the Asiatic and Pacific Species of *Mammea* L. (Guttiferae). *Communs. Forest Res. Inst., Bogor Nr.* **72:** 1-63.

——. 1969. *Kayea* Wall. and *Mesua* L. (Guttiferae). *Reinwardtia* **7:** 425-431.

Kundu, B. C., and B. Gupta. 1967. Parallel Venation in *Calophyllum inophyllum* Linn. *Sci. Cult.* **33:** 122-124.

Lanjouw, J. *et al.* (ed.) 1966. *International Code of Botanical Nomenclature* (Edinburgh, August 1964). Utrecht.

——, and F. A. Stafleu. 1964. *Index. Herbarriorum.* Part I (ed. 5). *Regnum Veg.* **31:** 1-251.

Lawrence, G. H. M. 1951. *Taxonomy of Vascular Plants.* New York.

Linne, C. 1753. *Species Plantarum.* (First edition). Stockholm. Vol. I. (facsimile ed. London, 1957).

——. 1754. *Genera Plantarum.* (Fifth edition). Stockholm.

Maheshwari, J. K. 1960. Taxonomic studies on Indian Guttiferae I. The Genus *Calophyllum* L. *Bull. bot. Surv. India* **2:** 139-148.

——. 1963. Taxonomic Studies on Indian Guttiferae II. The Genus *Mesua* Linn. *Bull. bot. Surv. India* **5:** 335-343.

——. 1964. Taxonomic studies on Indian Guttiferae III. The genus *Garcinia* Linn. s. l. *Bull. bot. Surv. India* **6:** 107-135.

Melchior, H. (ed.) 1964. A Engler's *Syllabus der Pflanzenfamilien.* 12th ed. II Band. *Angisopermen.* Berlin-Nikolassee.

Melville, R. 1969. Leaf Venation Patterns and the Origin of the Angiosperms. *Nature, Lond.* **224:** 121-125.

Planchon, J. E., . and J. Triana. 1860-1862. Mémoire sur la famille des Guttifères. *Ann. Sci. nat. Bot.* (*Paris*) **13:** 306-376, **160; 14:** 226-367., 1860; **15:** 240-319, 1861; **16:** 263-308, 1862.

——. 1861. Sur la famille des Guttifères. *Bull Soc. bot. Fr.* **8:** 26-29, 66-73, 96-100.

Prain. D. 1901. A new Assam timber tree. *Indian Forester.* **27:** 61-62.

Prakash, U. 1965. A survey of the fossil dicotyledonous woods from India and the Far East. *J. Paleont.* **39:** 815-827.

Robson, N. K. B. 1961. Guttiferae. In *Flora Zambesiaca.* London. **1:** 378-404.

Takhtajan, A. 1959. *Die Evolution der Angiospermen.* Translated by W. Hoppner. Berlin.

Vesque, J. 1893. Guttiferae. In DC *Monographiae Phanerogamarum.* Paris. **8:** 1-669.

Vestal. P. A. 1937. The significance of comparative anatomy in establishing the relationship of the Hypericaceae to the Guttiferae and their allies. *Philipp. J. Sci.* **64:** 199-252.

Wallich, N. 1832. *Plantae Asiaticae Rariores.* Vol. III. London.

Adv. Pl. Morph. 1972: 153-169

THE DEVELOPMENT OF THE FLOWER AND NECTARY OF THE DWARF CAVENDISH BANANA

BY A. FAHN AND M. KOTLER

Department of Botany, The Hebrew University, Jerusalem, Israel

ABSTRACT

The development of the ovarial part of the flower of the Dwarf Cavendish banana was described. Special attention was paid to the development of the nectary which consists of an epithelium lining a centrally situated cavity. In the female flower this cavity enlarges and appears three-lobed in cross-sections of the upper portion of the ovary. The lobes are narrow and penetrate between the three carpels. In the male flower the nectary is complicately branched, occupies the entire length of the aborted ovary as well as the space in which locules appear in the female flower. In both the male and female flowers the nectary cavity has three slit-like ducts which open at the top of the ovary, near the base of the style. The tall cells of the nectary epithelium divide transversally during development and produce 4-7 celled uniseriate hairs. The cells of the nectary epithelium, which in the mature flower are covered by a thick layer of mucilage, break down after nectar secretion.

The result of the study of the arrangement and structure of the vascular bundles in the ovarial part of the flower supports the view that the free tepal belongs to the inner whorl of the perianth and does not represent the sixth stamen.

INTRODUCTION

The flowers in the banana inflorescence are arranged in clusters, usually termed "hands". Each hand is borne on an obliquely transverse prominence ("cushion") subtended by a spathe-like bract. The individual flowers are ebracteolate.

The basal part of the inflorescence consists of up to 20 or 30 (in the Dwarf Cavendish banana 5-15) hands of female flowers. Above them there is a number of hands with neutral flowers. The remainder of the inflorescence, which continues to develop until the shoot above the ground dies, consists of many hands of male flowers.

The structure of the mature flower is as follows (Simmonds, 1959):—The perianth is zygomorphic and consists of two structures, the abaxial compound tepal, which has three big and two alternately placed small lobes, and an adaxial free tepal. The androecium consists of five stamens or staminodes. However, male flowers with six stamens have occasionally been observed by us. The missing member of the supposed complement of six is missing from the adaxial position i.e. opposite the free tepal. The usual interpretation of the flower is that the perianth is six-membered, the inner whorl being composed of the two small lobes of the compound tepal and the free tepal, and that the androecium consists of two whorls

---

We are indebted to Dr Ella Werker for help in the preparation of the manuscript.

of three, reduced to five by loss of one stamen. A different view is that the perianth consists only of one whorl of three members and that the free tepal represents the sixth stamen (Figs. 1, 2).

Male and female flowers differ in the following features: In the female flower the inferior ovary is well developed, and the style is massive. The stamens are usually reduced to staminodes, although functional stamens sometimes occur. In the male flowers the ovary is abortive, the style and stigma are slender, and the anthers are well developed. In the ovaries of both types of flowers there is a well developed nectariferous tissue.

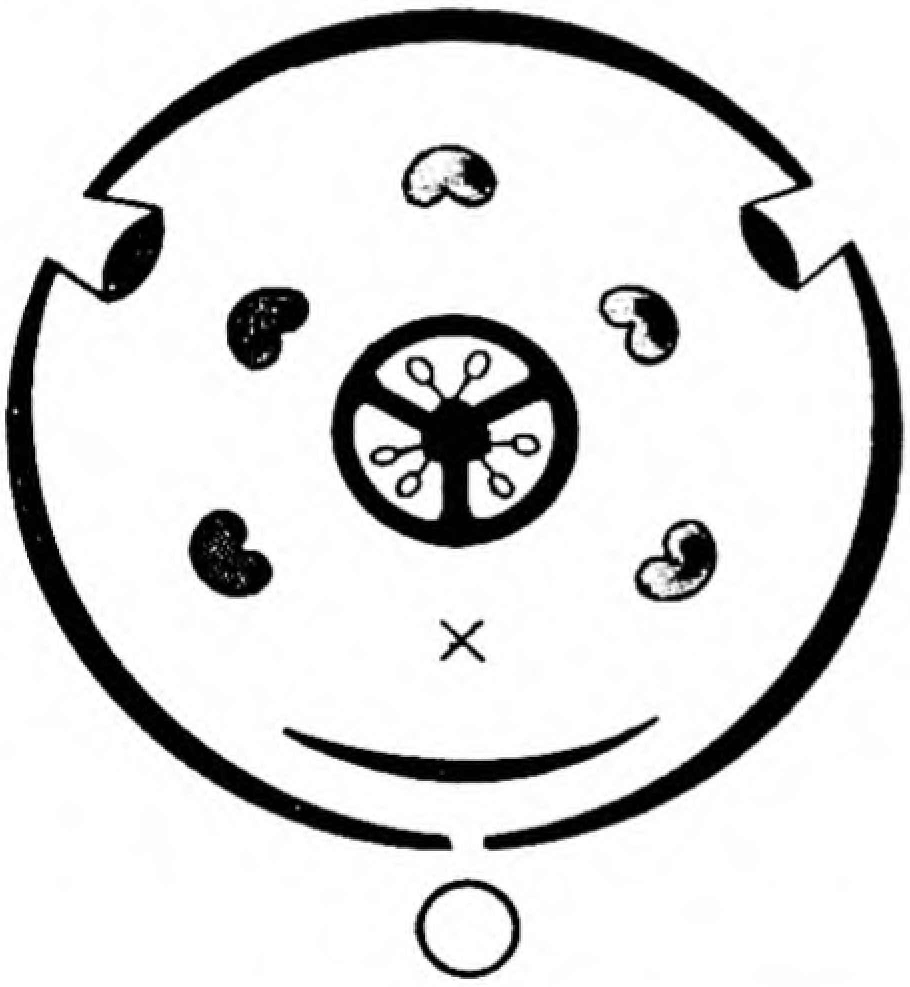


Fig. 1.

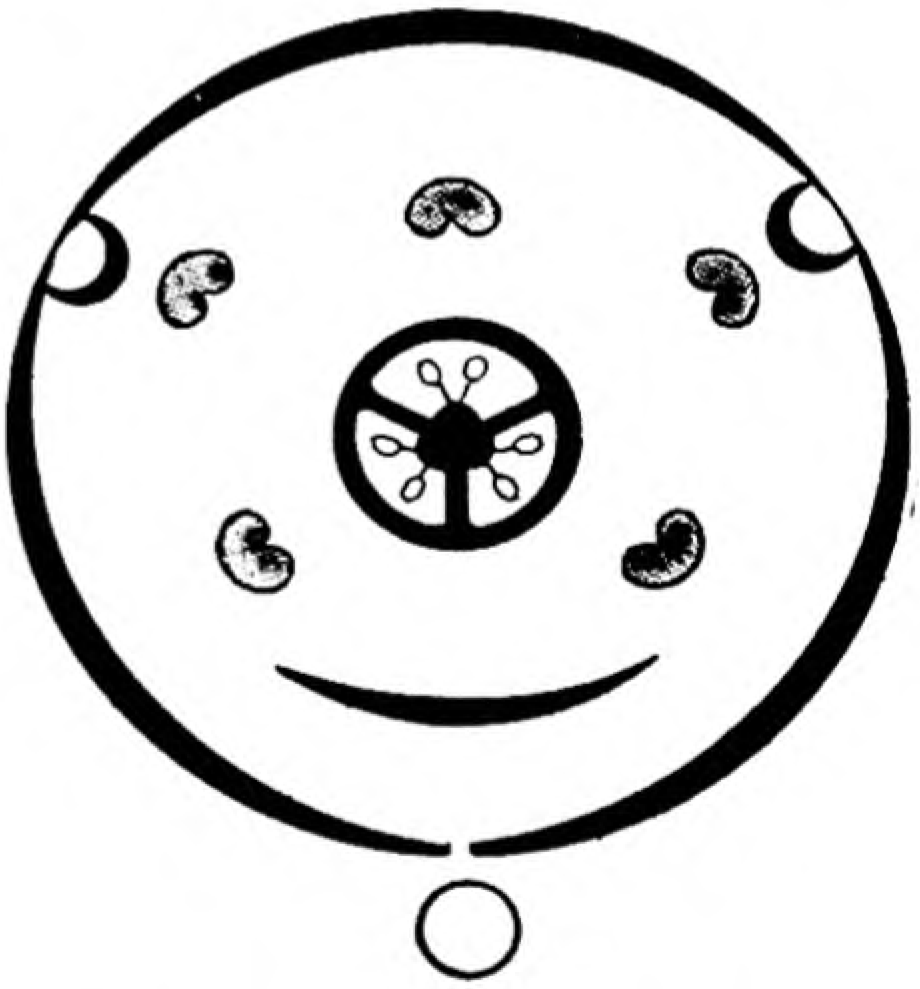


Fig. 2.

Figs. 1, 2. Floral diagrams, showing the two interpretations of the banana flower (adapted from Simmonds, 1959).

The flower primordia in the two tangential rows of each hand of the banana inflorescence arise in an alternating sequence, one in the adaxial being followed by one in the abaxial row (Fahn, 1953). According to White (1928) the initiation of the flower appendages is as follows: The young flower primordium is at first broadly dome-shaped. Then a depression begins to appear on the tip. The margins around the depression form the primordia of the outer tepals. Later, primordia of the two outer stamens and the free tepal develop. The third stamen of the outer ring is formed opposite the abaxial tepal. The two lateral tepals of the inner ring are initiated as folds inside the notch between the alternate outer ones.

Our observations were confined to the development of the ovule-bearing ovary of the female flower and the abortive ovary of the male flower of the Dwarf Cavendish banana. Special attention was paid to the correlation between the nectary and the locules.

The problem of the origin of the free tepal was studied by examining the number, shape and pattern of vascular bundles supplying the various flower appendages.

MATERIAL AND METHODS

Flowers at various stages of development were collected from plantations in the upper Jordan Valley and in the coastal plain of Israel. As the inflorescences with the flower primordia and the young flowers are hidden in the pseudostems, these had to be dissected in order to obtain the flowers of the various development stages. The flowers were fixed in FAA and embedded in paraffin. Serial cross-sections, 10μ thick, made with a microtome, were stained with safranin and fast green. Some longitudinal sections were also prepared.

OBSERVATIONS

The distinction between female and male flowers at their initial stages can be made only by their position on the inflorescence. Therefore, very young developing flowers of the lowest hands (female) were obtained from inflorescences hidden deep at the base of the pseudostem. The very young male flowers were taken from developing hands at the top of the inflorescence which had already reached a somewhat higher position in the pseudostem. When the inflorescence is 6-7 mm long (Stoler, 1960) the two sexes of the flowers can be morphologically distinguished by the big difference between the length of the ovaries. At such a stage, however, there is no difference between the female and neutral flowers (cf., Alexandrovicz, 1955; White, 1928).

Development of the ovary of the female flower.—Female flowers were chosen from six main successive stages of development for preparation of serial cross-sections. Sections of three critical levels of the developing ovarial part of the flower (L 1— at about half the height of the ovary above its base: L 2— about 3/4 of the height of the ovary: L 3—close to top of ovary) were described for each of the stages chosen.

Stage 1.—The youngest stage examined: The maximal width of the ovarial parts was 0.72 mm.

L 1.—In the centre there is a triangular cavity the walls of which are convex and covered by an epithelium of approximately cubical cells (cf. Fig. 3 C). The anticlinal walls of these cells are thinner than the periclinal ones.

L 2.—In the cavity, six angles and six shallow uneven lobes can be distinguished (Fig. 3 A). The lobes represent the edges of the three carpels. In the epithelial cells anticlinal cell divisions can be seen (Fig. 3 C). In the neighbouring parenchyma cells divisions in various planes occur.

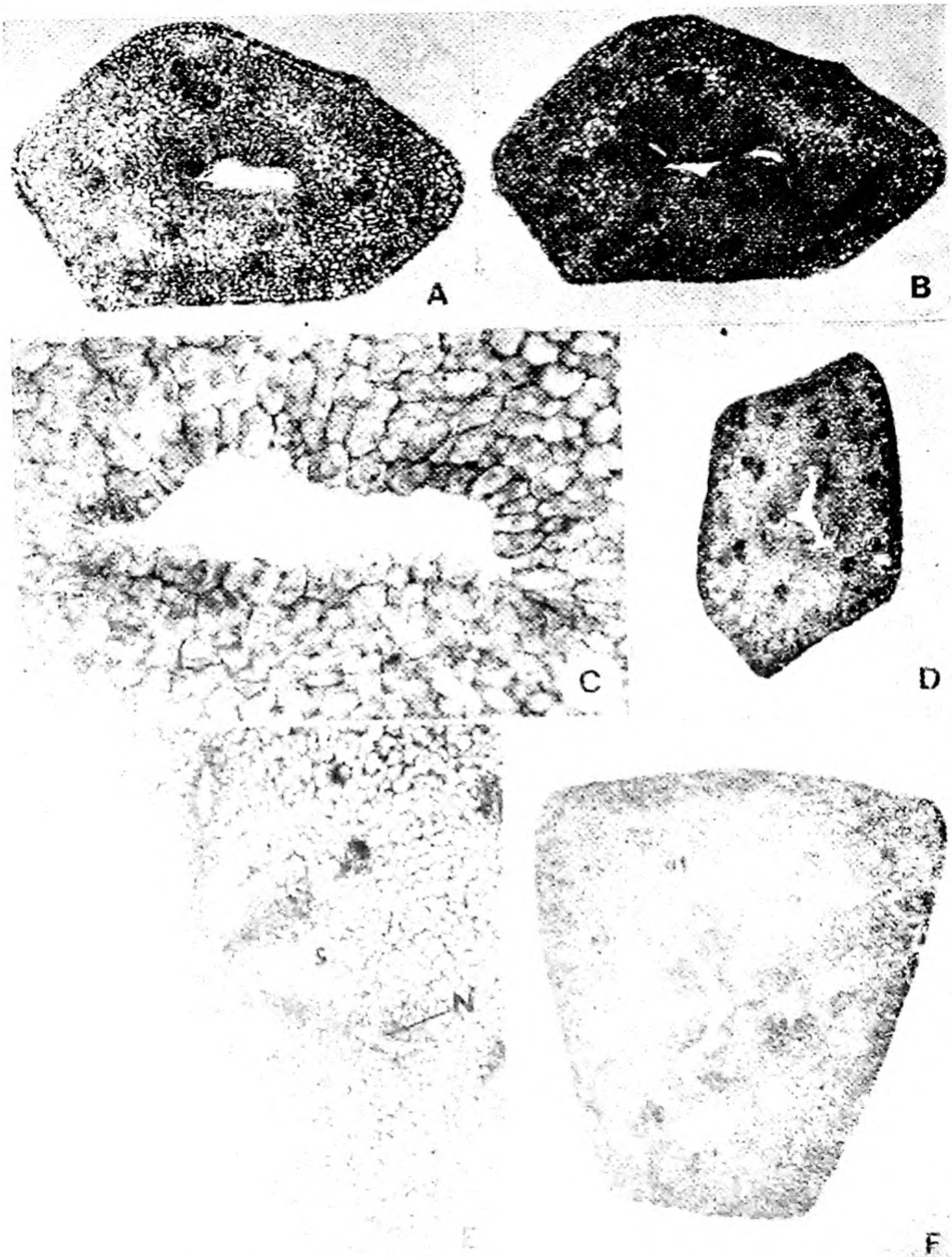
L 3.—The cavity exhibits several elongated narrow branches (Fig. 3 B). These branches are the continuations of angles seen at the lower levels.

Stage 2.—The maximal width at the ovarial part was 1.45 mm.

L 1.—The central cavity consists of 3 rays which widen at their ends in the shape of crescents (Fig. 3 D). These crescents represent the future loculi. The walls of the rays represent the carpellary margins. The crescent-shaped cavities are lined by an organized epithelium, whereas the central part is lined by irregularly arranged cells. In the tissue surrounding the radial cavities relatively many cell divisions take place.

L 2.—The median portions of the radial cavities become closed and appear as sutures. The outer ends of the radii representing the locules are still open. In the centre a relatively large cavity with 6 angles is present. Three of the alternating angles are continuous with the radial sutures. The sutures consist of two rows of tall epithelial cells (cf. Fig. 3 E). The epithelium lining the central cavity, which will develop into the nectary, consists of irregularly arranged cells.

L 3.—At this level there are six radial sutures (Fig. 3 E). Three are continuous with



[illegible] of female flowers at various stages of development and [illegible] L 3, x 120. C. Stage 1, L 2, x 530. D. Stage 2, L 1, [illegible] at the distal ends of which slit-like cavities may be [illegible] (L) and alternating with them there are three nectary duct (N) [illegible] the sutures of L 2 and at their distal ends very narrow cavities (loculi) are seen. The other three sutures, alternating with them, end with slits which will develop in mature flowers into ducts through which the nectar is exuded.

Stage 3.—The maximal width of the ovarial part was 1.71 mm.

L 1.—Similar to L 1 of Stage 2 but the locules are more developed and one radial cavity is closed (Fig. 3 F). The epithelial cell shapes range from cubical to elongated perpendicular to the ray. The epithelium lining the outer wall of the crescent shaped locule consists of flattened cells (Fig. 4 A). In the sutures the epithelial cells are tall (Fig. 4 B).

L 2.—All three rays are closed, the loculi are open and in the centre there is the cavity which will develop into the nectary (Fig. 4 C).

L 3.—Six slits, of which three belong to the future nectary and three of which represent the future locules are seen at this level.

Stage 4.—The maximal width of the ovarial portion was 2.14 mm.

L 1.—The three locules have the shape of crescent shaped cavities. The adaxial walls of the locules became concave as a result of a considerable growth in height of the epithelial cells, especially in the region of fusion of the carpel margins (Fig. 5 A). These cells divide later periclinally and a multiseriate epithelium of relatively tall cells, which take up a great part of locule cavities, is formed. The epithelium of the abaxial walls of the locules consists of cubical cells. Along the sutures there are two rows of tall cells. Such cells line also the very small central cavity where the three sutures end.

L 2.—The three locules appear as very narrow small crescents. In the centre of the ovary the developing nectary has a three armed star-like cavity. Alternating with the nectary arms there are the three sutures of the carpels (Fig. 5 B, C; Fig. 6 A).

L 3.—The nectary arms appear as narrow slits distant from the centre. Somewhat below the very top the slits may still be connected to the central cavity by closed sutures. These slits serve as ducts connecting the nectary with the surface of the ovary around the base of the style. The locules, which also become slit-like, converge towards the centre when approaching the top of the ovary and enter the style (Fig. 6 B).

Stage 5.—Here several flowers were examined and described. The maximal width of the ovarial portion was 3.54 mm.

L 1.—The locules have acquired the shape of a horseshoe (Fig. 6 C) as a result of divisions of the cells on the abaxial side the further growth of the tall cells lining the adaxial side of the locules (Fig. 6 D). The tall cells divide anticlinally and form mucilage secreting hairs, which are specially developed at the place of fusion of the carpel margins (placentae) (Fig. 6 C). With the cessation of growth of the convoluted margins the whole epithelium covering them is of a uniform structure, and obtains, with time, the appearance of a dense brush of mucilage secreting hairs (Fig. 6 C).

From the extreme edges of the convoluted carpels the ovules develop between the hairs (Fig. 7 A).

With further growth the stellate parenchyma of the ovary wall changes into an aerenchymatous tissue with large air spaces. These spaces develop schizogeneously (Fig. 7 A, B).

In the centre of the ovary there is a cavity in continuation with the nectary which is developed in the higher levels of the ovary. The cubical cells of the epithelium of this cavity contain dense protoplasts.

L 2.—The central cavity of the nectary has

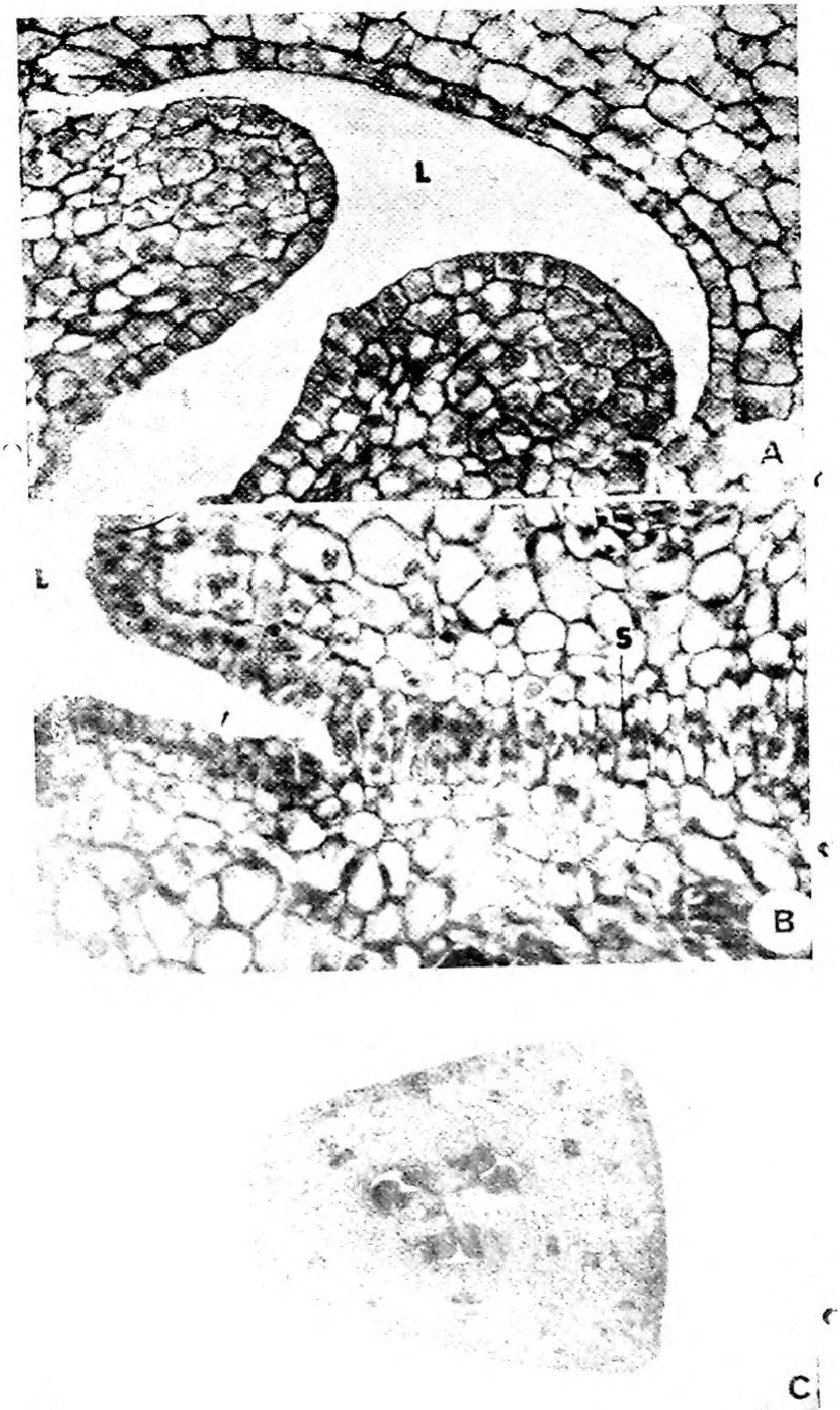


Fig. 5. A–C. As in Fig. 3. A. Stage 2, L. 4, a portion of Fig. 3 F, showing one crescent-shaped [illegible] (L) x 530. B. Stage 3, L. 1, showing a [illegible] (S) formed by closing of carpellary margins, x 530. C. Stage 3, L. 2, showing a small nectary [illegible] in the centre, x 40.

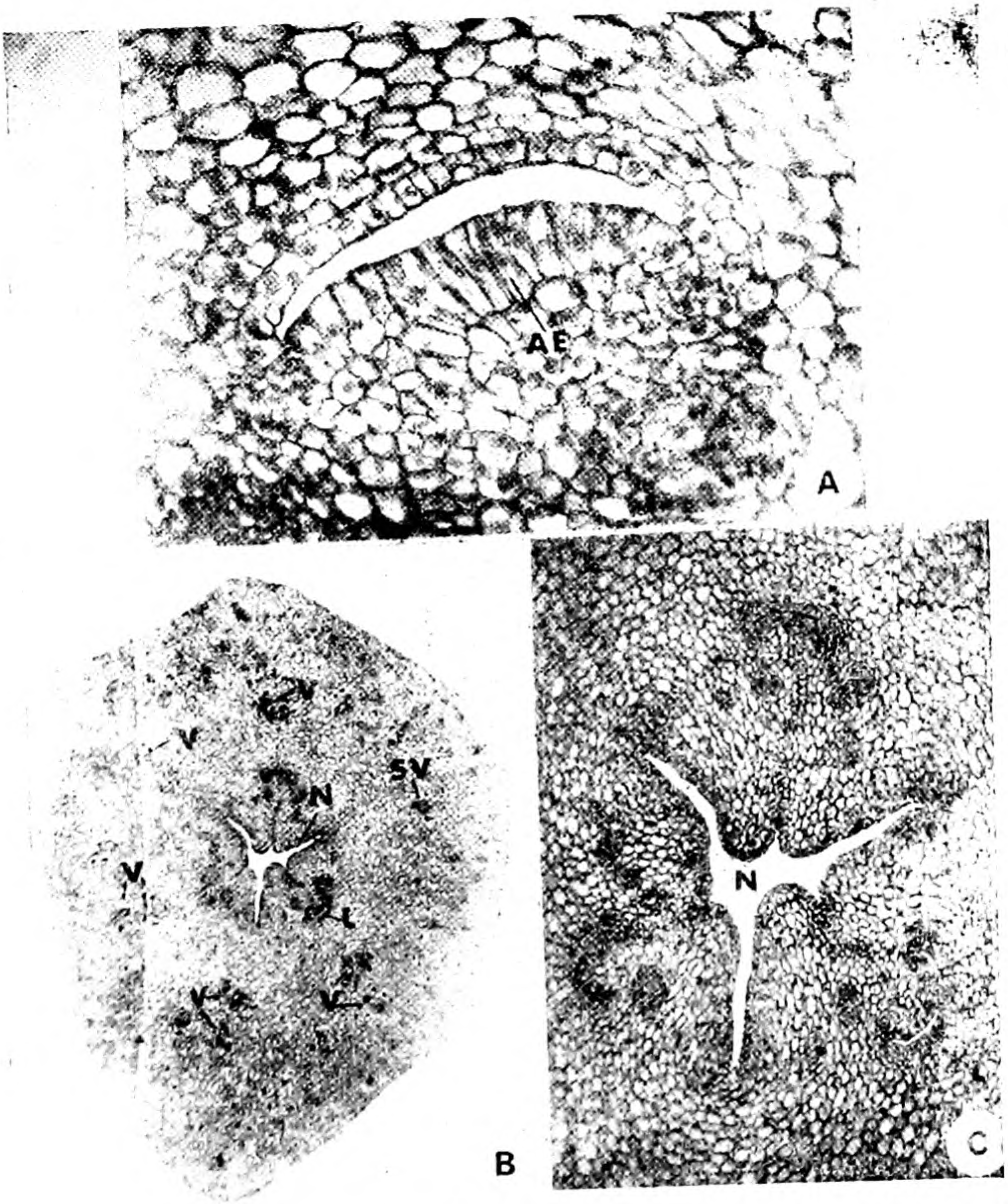
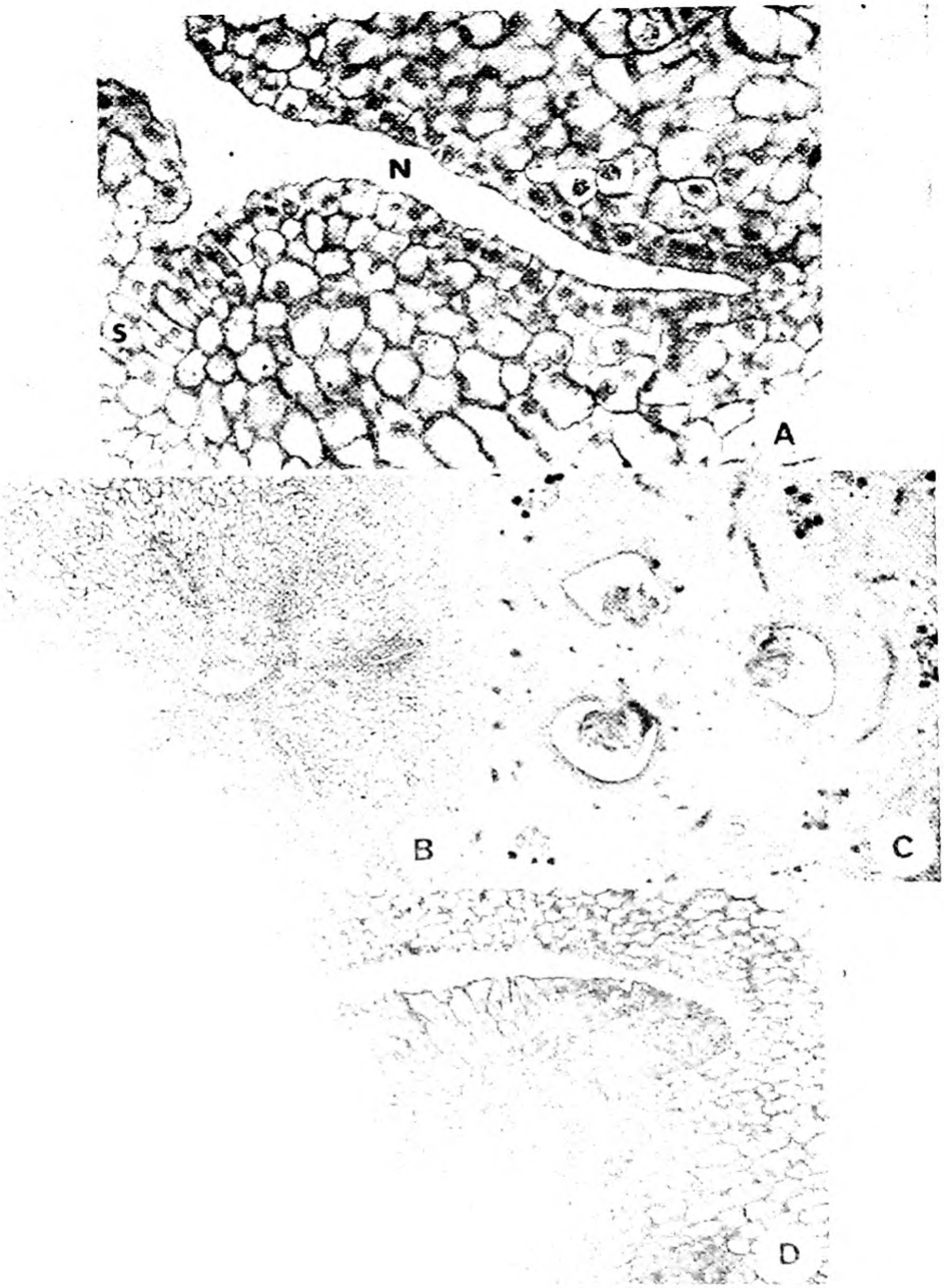


Fig. 5 A—C. As in Fig. 3. A. Stage 4, L 1, showing a locule, the adaxial epithelium of which consists of tall cells, x 530. B. Stage 4, L 2, showing 5 pairs of vascular bundles opposite the three locules and opposite two of the arms of the nectary. Opposite the third nectary arm there is a single vascular bundle SV, x 40. C. Central portion of B, x 120.

AE, adaxial epithelium; L, locule; N, nectary; SV, vascular bundle; V, vascular bundles.



[illegible] arm (N) and a portion of a suture (S) leading [illegible] three of which represent the continuation of the loc[illegible] the nectary ducts which lead to the top surface of the ovary [illegible] horseshoe V-shaped locules. At the placental region there are mucilage [illegible] showing a locule, in the placental region of which the tall cells have [illegible] secreting cells. × 130.

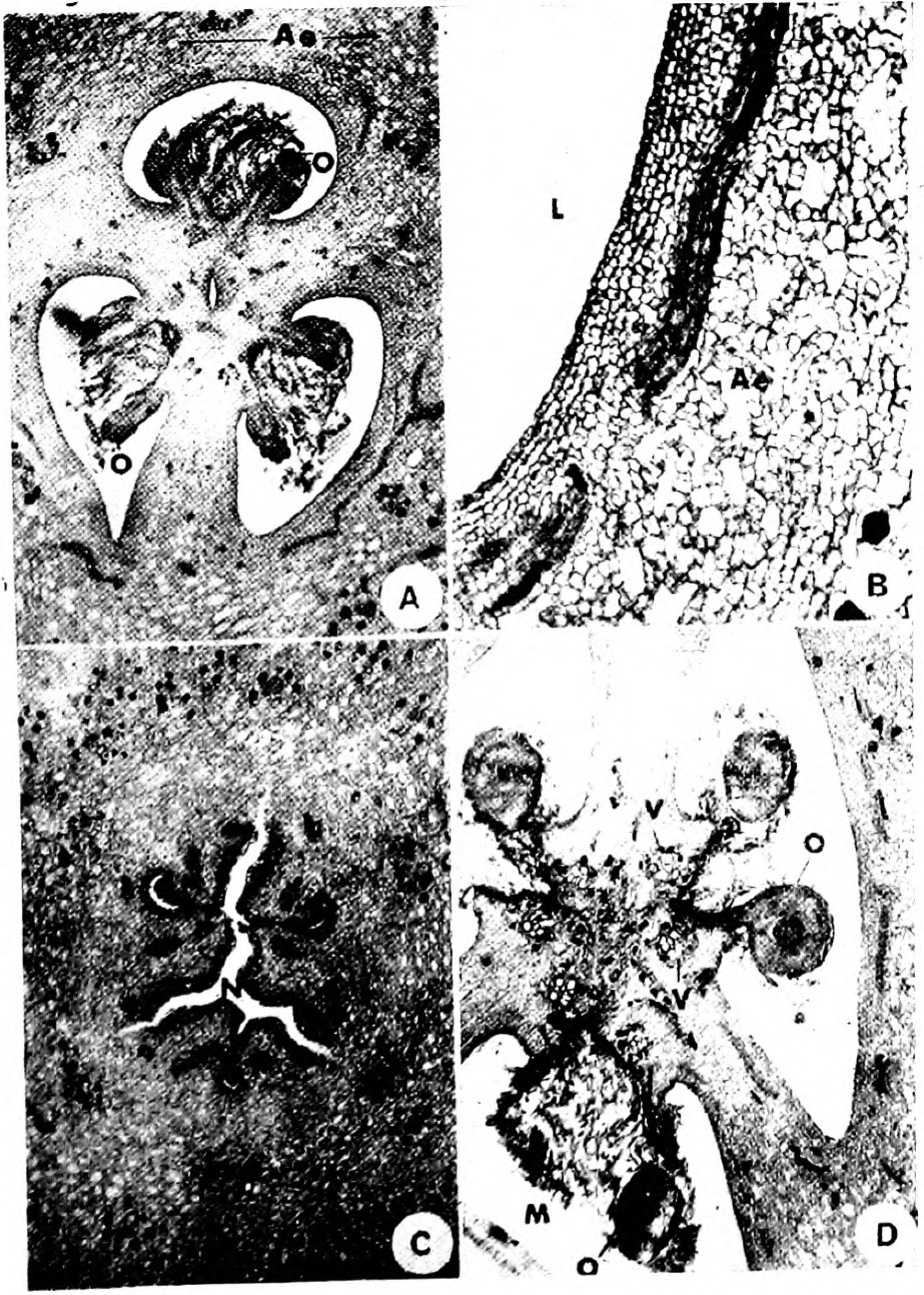


Fig. 7 A-D. As in Fig. 3. A. Stage 5, L 1, showing developing ovules and aerenchymatous tissue in the ovarial wall, x 50. B. Portion of A, showing several layers of compact parenchyma lining the abaxial side of the locule and the aerenchymatous tissue neighbouring it. x 120. C. Stage 5, L 3, central portion of ovary showing nectary and locules, x 40. D. Stage 6, L 1, central portion of ovary showing ovules, mucilage secreting cells, and three pairs of ventral vascular bundles, x 40.

Ae, aerenchymatous tissue: L locule: M, mucilage secreting cells; N, nectary; O, ovule; V, vascular bundles.

3 angular lobes which elongate gradually at higher levels of the ovary. This cavity is lined by tall epithelial cells which will constitute, in the future, the secretory epithelium of the nectary. The sutures can be distinguished only close to the cavity of the nectary and near the locules.

L 3.— The nectary lobes are large and crooked and somewhat branched, especially at the sutures. The loculi are very small and are connected to the nectary by the sutures (Fig. 7 C).

Stage 6.—Flowers on an inflorescence just before, or at the very stage of emergence from the pseudostem. The ovary has already grown considerably in volume and the locules occupy most of it. In this stage several flowers of closely successive ages were examined.

L 1 — The ovules lifted on funiculi are surrounded by mucilage secreting hairs (Fig. 7 D).

The central cavity which was evident at this level in the earlier stages of development [illegible] here completely. The [illegible] an epithelium of some-[illegible] cubical cells except for [illegible] the mucilage secreting [illegible]

[illegible] ules are reduced [illegible] thick and the [illegible] into an irregu-[illegible]. The main [illegible] level occu-[illegible] examining [illegible] levels of [illegible] nd [illegible] of the [illegible]

The nect[illegible] is [illegible] tall cells. At [illegible] epithelial cells occur [illegible] the cytoplasm is [illegible] Later, this regular [illegible] nuclei becomes disturbed [illegible] cell divisions take place (Fig. 8 C). Hairs, 4-7 cells long, are formed (Fig. 8 D). All these cells are filled with a dense cytoplasm.

L 3.— The nectary is three armed. At higher levels the arms become detached from the centre and they form three slits which have moved towards the periphery and which finally open to the receptacular surface as grooves imbedded between the furrows of the style and the stamens. One nectary opening usually appears between a stylar furrow and the free tepal. The locules, which here become very small and ellipsoidal, move towards the centre. The nectarial epithelium at this level is flat.

The mature flower.—The ovary structure described above, belongs to female flowers of an inflorescence which was at the stage of emergence from the pseudostem. This structure does not change substantially after opening of the bracts of the hands and opening of the flowers which enlarge in size. In the opened flowers the nectary epithelium is covered by a thick layer of mucilage which contains nectar secreted by the cells. Later, the epithelial cells break down and their whole content is released into the cavity of the nectary (Fig. 9 A-D).

Fruit pulp development.—As is well known, the development of the fruits of the Dwarf Cavendish banana is parthenocarpic. Simmonds (1959, p. 30) suggested that this is achieved by an autonomous stimulus of growth which replaces a stimulus, that, in the seeded banana, is derived from the developing seeds.

The fruit pulp develops from 3-5 layers of parenchyma cells situated between the epithelium of the locules and the aerenchymatous tissue (described in Stage 5, Fig. 7 B). The cells of these layers divide (Fig. 10 A) and then distinctly elongate in the radial direction of the fruit (Fig. 10 B), and produ

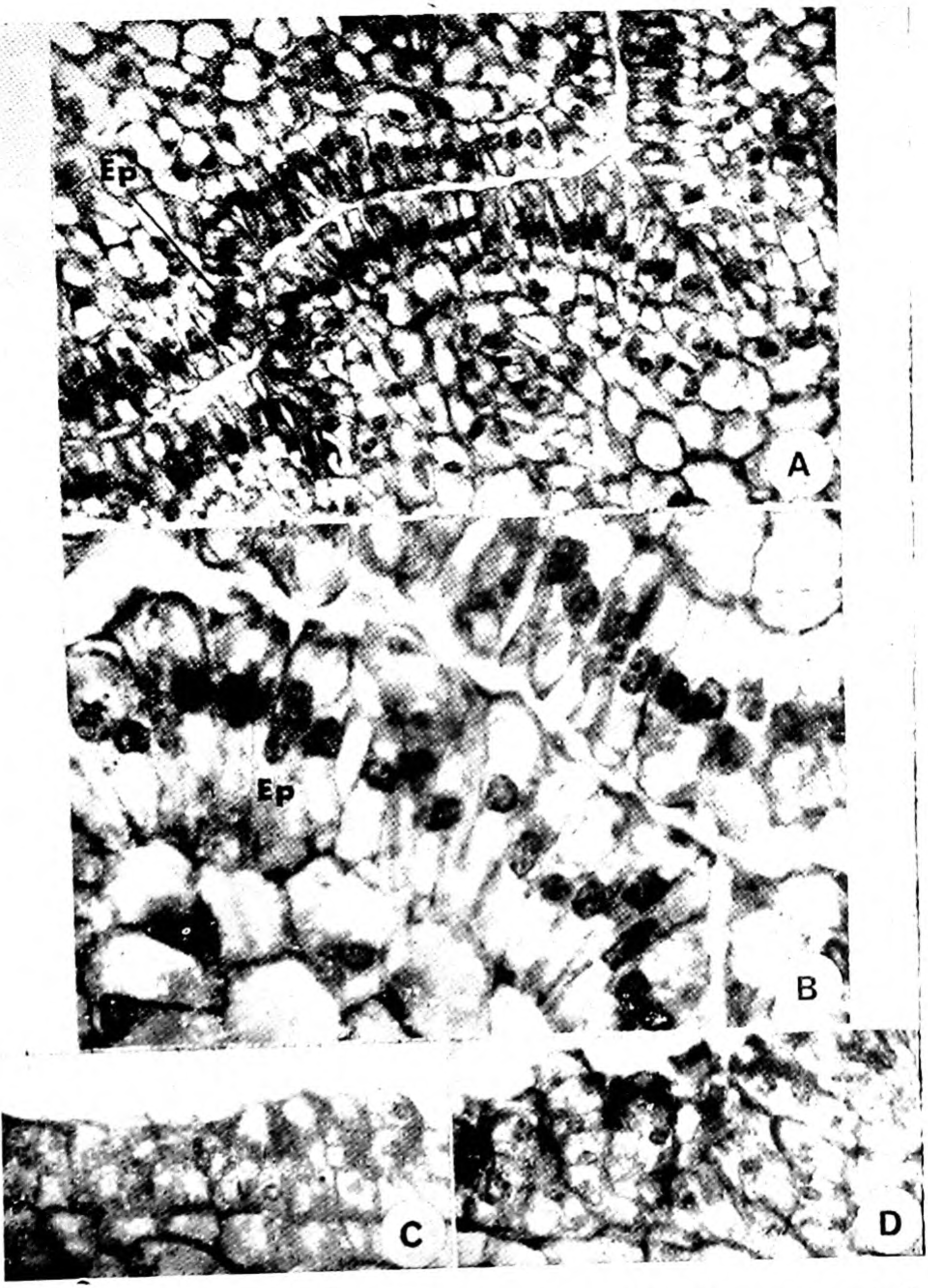


Fig. 8 A-D As in Fig. 3. Stage 6, L 2, showing nectary epithelium (Ep) consisting of tall cells. A, x 500, B, x 1200. C, D. Stage 6, L 2, showing successive stages of hair development from the tall epithelial cells of the nectary as a result of transversal divisions, x 500.

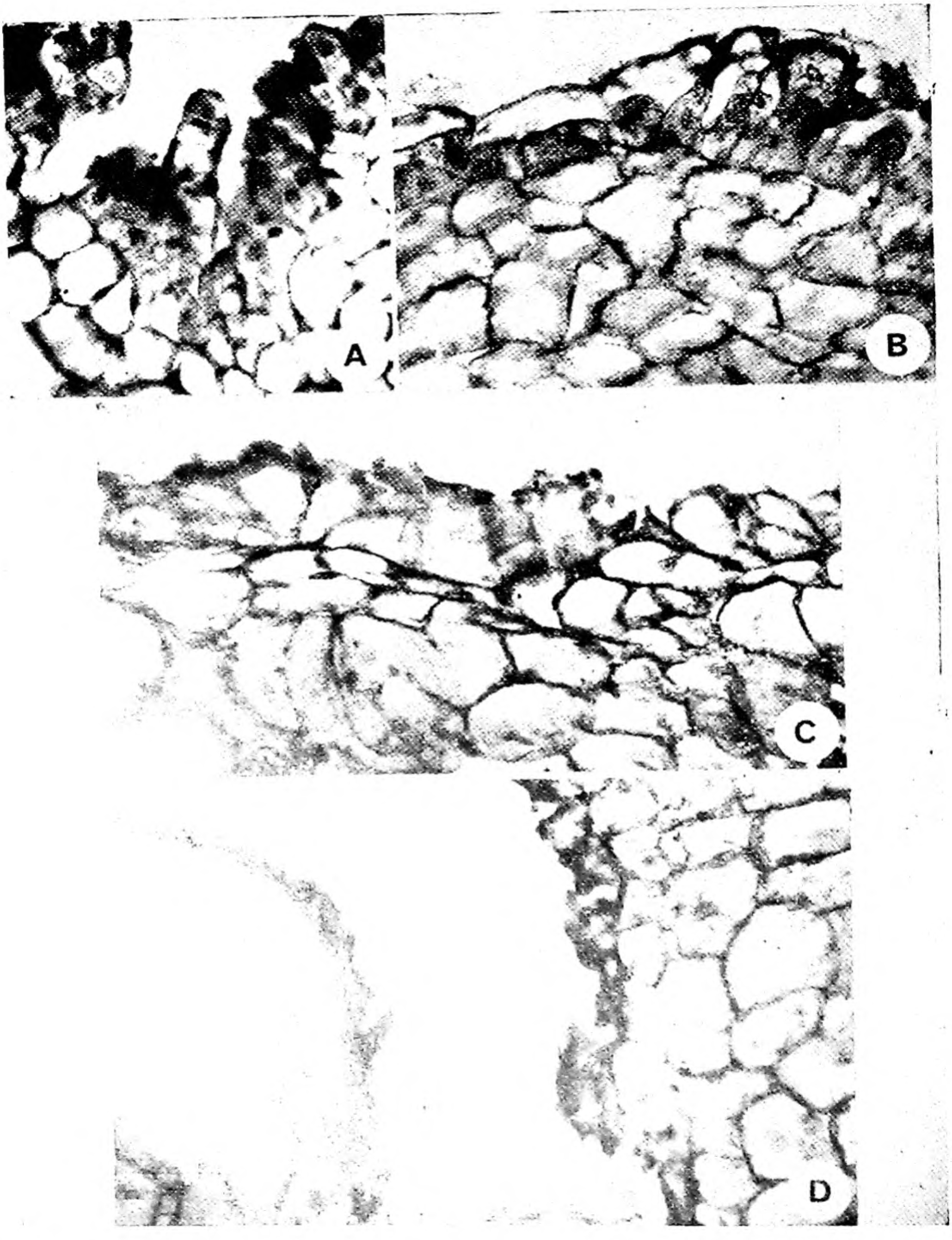


Fig. 9 A-D. Cross-sections in the region of the nectary of flowers showing various stages of disintegration of the epithelium associated with nectar secretion, x 500.

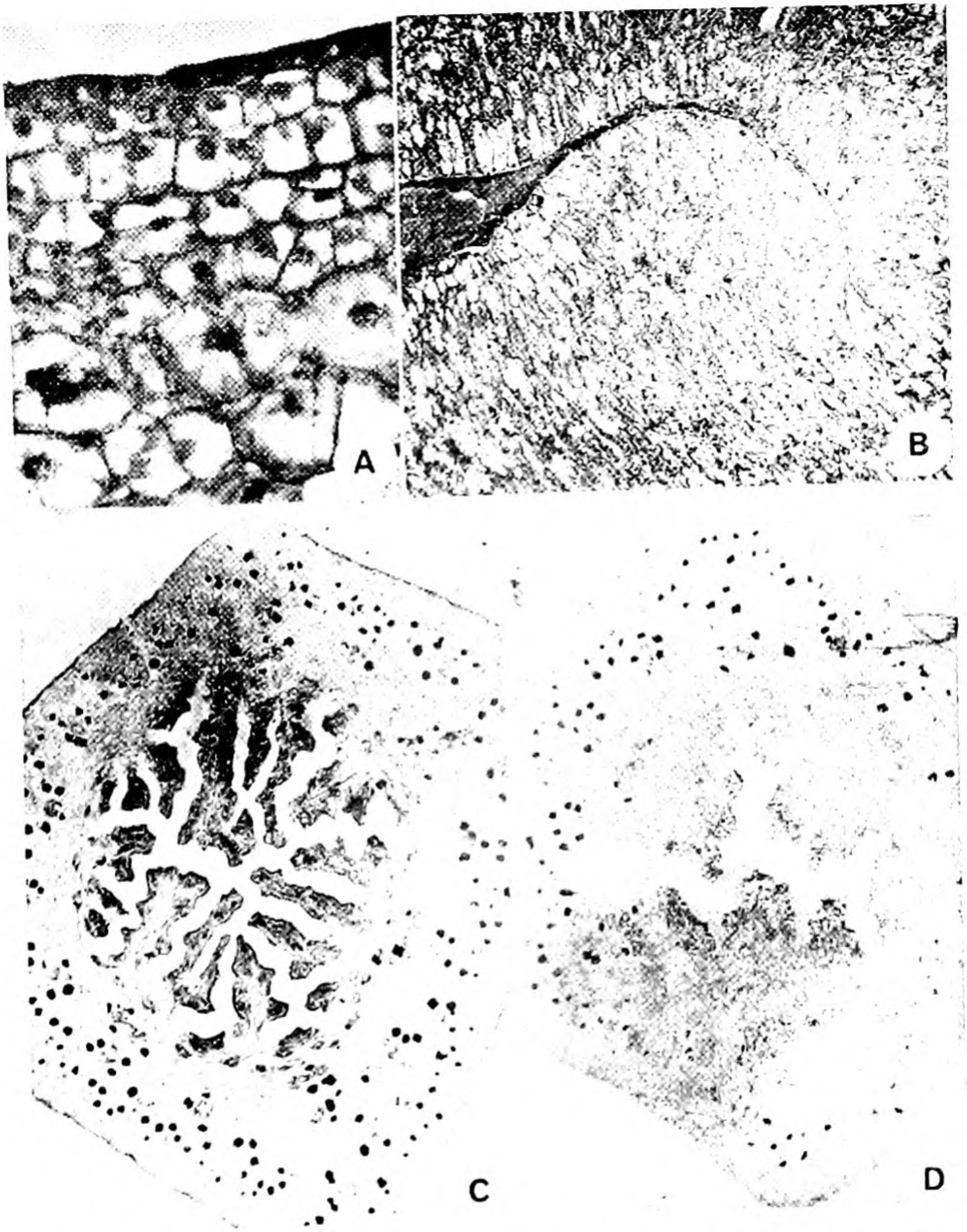


Fig. 10 A-D. A. Early stage of fruit pulp development, showing immediate results of cell divisions, x 500. B. A later stage of pulp development, showing radially elongated cells filled with starch grains x 100. C. A cross-section of the ovarial part of an almost mature male flower at its middle height, showing the well developed labyrinth of nectary canals, x 40. D. As in C but at a higher levels of the ovarial portion, x 75.

has the pulp. Most of the planes of division here are periclinal, but anticlinal ones also occur (Fig. 10 A) The divisions in the epithelial cells of the locules are anticlinal. The process of growth of the pulp region continues until it occupies most of the fruit. Its large cells are elongated and arranged radially around each locule. They are filled with starch. Starch grains occur also in the aerenchymatous tissue. The fruit, although it develops from an inferior ovary, can be regarded as a berry, the exocarp of which consists of the outer epidermis and the aerenchymatous tissue, the mesocarp which consists of the pulp, and the endocarp which is limited to the epithelium of the locule.

The ovarial portion of the male flower.—The male flowers which are borne on hands situated on the upper part of the inflorescence, continue to initiate and develop even after the female flowers reach maturity.

In the mature male flowers a labyrinth of [illegible] occupies the entire length of [illegible] ovary and also the space [illegible] in the female flowers [illegible] end the labyrinth [illegible] three lobes of [illegible] ducts (Fig. 10 D; [illegible] three grooves [illegible] similar to that [illegible]

[illegible] of the [illegible] the de- [illegible] there [illegible] gives [illegible] devel[illegible] many times [illegible] of canals is [illegible] histological specializat[illegible]

Vascularization of th[illegible]—In the centre of the ovary of th[illegible] flower there are three pairs of vasc[illegible] bundles (Fig. 7 D). Each pair of these bundles represents the ventral bundles of one carpel. Outside each locule there is a pair of vascular bundles arranged radially in the median plane of the carpel (Fig. 5 B). The distance between the bundles of each pair is small at the base of the ovary and increases towards the top of the ovary. At the top, each of the three adaxial bundles splits in two; one representing a dorsal trace of the carpel, enters the style, and the other enters one of the three outer stamens. The three abaxial bundles are tepal traces. The bundles of the two inner stamens appear opposite two septa. At the periphery of the ovarial portion six distinct vascular bundles represent the median tepal traces (Fig. 5 B).

In the male flower the ventral traces of the carpels are missing. At the top of the ovarial part (seen in Fig. 11 A) the tepal bases are already partly separated from the ovary. Here, the three dorsal traces are already far apart from the corresponding traces of the outer stamens and vascular bundles situated opposite two nectary lobes enter the two inner stamens.

In order to learn whether the free tepal represents one of the three members of the inner whorl of the perianth or the sixth missing stamen, developing male and female flowers with the usual number of 5 stamens and some female flowers with 6 stamens were sectioned and examined.

The examination of sections revealed the following:—in the ovarial part of the male and female flowers with 5 stamens, groups of two radially arranged vascular bundles appear opposite two of the three nectary lobes. The inner ones of these two groups supply the two inner stamens, whereas the outer enter the compound tepal. Opposite the third nectary lobe appears only one bundle which enters the free tepal. In flowers which had 6 stamens the position opposite the third nectary lobe was similar to that opposite the two other lobes, i.e., two radially arranged bundles occurred, the inner of which entered the sixth stamen. In the flowers with 5 stamens the inner bundle lacks throughout the whole ovarial part.

The structure of the inner vascular bundles opposite the nectary lobes, whether two (in case of 5 stamens) or three (in case of 6 stamens) differs from that of the outer vascular bundles, including this entering the free tepal. The last mentioned bundles are simple collateral bundles with the xylem facing the centre of the ovary (Fig. 11 B). The bundles entering the inner stamens have the appearance of two collateral bundles fused tangentially in such a way that the xylem obtains a median position and the phloem groups are situated on two sides of the "fused" xylem (Fig. 11 B).

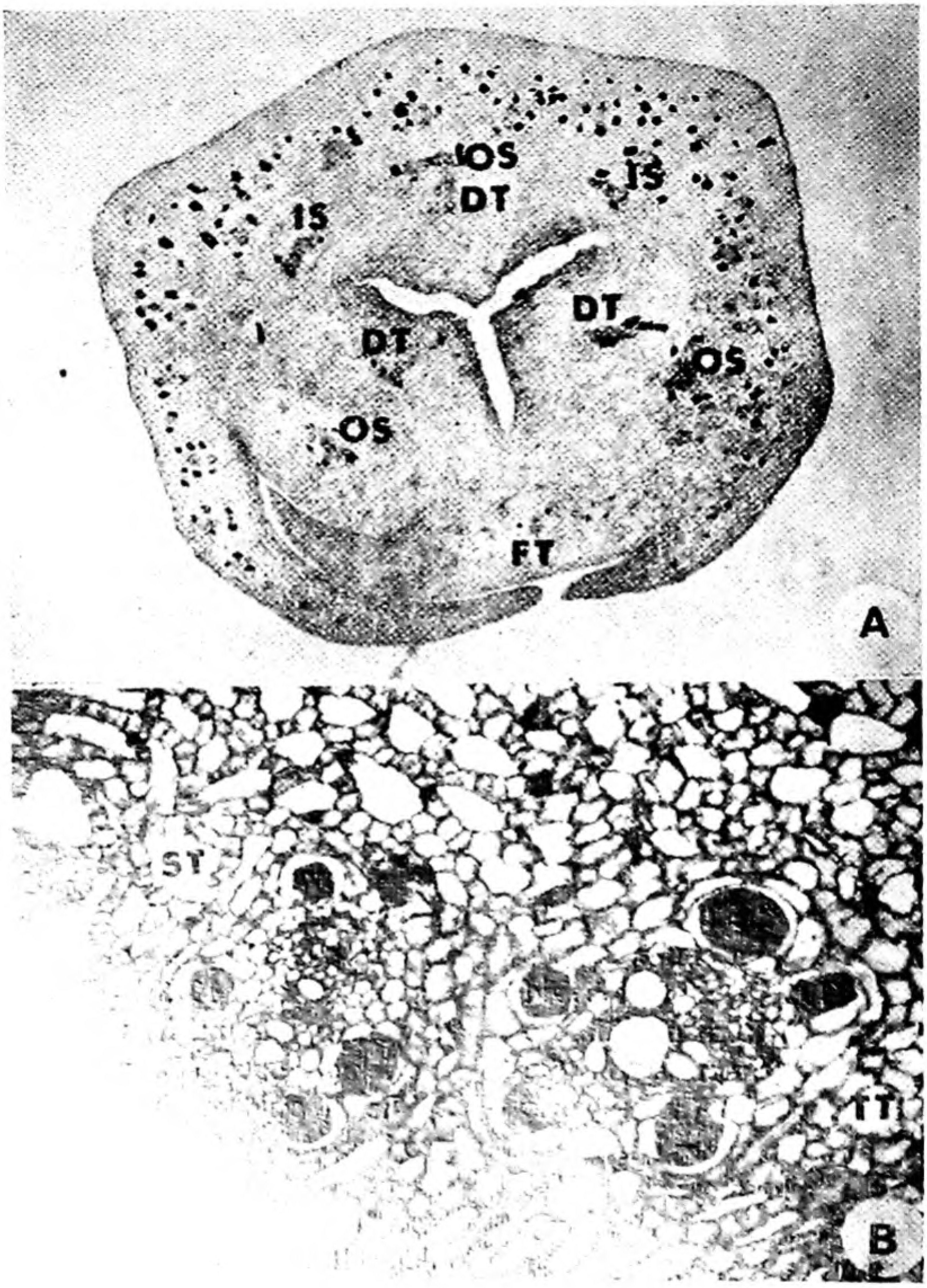
DISCUSSION

The alternating sequence of the development of the flowers of the two rows in the hands of the banana inflorescence was shown by Fahn (1953). He has based his conclusions on the pattern of differentiation of the flower primordia on the cushion of the hand and on the way the vascular system differentiates in later stages of the hand development. Ram et al. (1962) have suggested that the flower primordia arise simultaneously in both rows of a given hand. It should, however, be mentioned that the view expressed by Ram et al. is based on the impression received by comparing the various flower primordia of a given hand in which all the primordia have already developed. Observations made while carrying out the present work strengthened the previous conclusions of Fahn (1953).

The main aims of the present work were: (i) to study the ontogeny of nectary in the flowers of the two sexes and (ii) to check the two interpretations of the flower structure by means of the study of the vascular bundles.

White (1928) gave a general description of the development of the banana flower. According to our investigation carried out on the Dwarf Cavendish banana, and White's observations made mainly on the Gross Michel banana, the ovarian cavity is originally triangular in cross-section at lower level and six-lobed in the upper part. Later, with the partial fusion of the three carpels this cavity, which is lined with a glandular epithelium, is continuous except for the places of complete fusion of the placentae. The cavity lined by the glandular epithelium represents the nectary which opens at the top of the ovary via three slit-like ducts. In the female flowers the portion below the nectary elongates to form the ovary proper (cf. Fahn et al., 1961). In the male flower the nectary is much more developed and occupies most of the volume of the ovarial part of the flower (cf. Baumgartner, 1913). The male banana flowers secrete much more nectar than do the female ones (Fahn, 1949). The tall epithelial cells of the nectary divide and produce hairs 4-7 cells long. At the time of nectar secretion the epithelium is covered by a thick mucilage layer. Later the epithelial cells break down. The exact process of nectar secretion by the epithelial cells and the question of whether, after their breakdown, the cell layer next to them is capable of secreting nectar, are not yet clear.

There are two interpretations of the banana flower (cf. Simmonds, 1959). According to one interpretation (Fig. 1) the abaxial compound tepal consists of


OS
DT
IS
IS
DT
DT
OS
OS
FT
A
ST
TT
B

[illegible] of an almost mature male flower at the top of [illegible] three lobes, x 40. B. A pair of radially arranged vascular [illegible]

DT, [illegible] IS, traces of the two inner stamens; OS, traces of the three outer [illegible] sepal trace.

three major tepals of the outer whorl and of two minor tepals of the inner whorl. The large adaxial free tepal represents the third tepal of the inner whorl of tepals. The androecium consists of three outer and two inner stamens. In view of this interpretation the sixth stamen (the third of the inner whorl), that opposite the free tepal, was lost.

According to the second interpretation (Fig. 2) the perianth consists of one whorl of three members only, the two minor lobes being morphologically insignificant, and the free tepal represents the sixth stamen.

Our observations made on the vascular bundles in the inferior ovary of developing flowers revealed the occurrence of three pairs of bundles opposite the three locules, two pairs opposite two nectary lobes and a single bundle opposite the third nectary lobe. The 5 outer bundles of the 5 pairs enter the abaxial compound tepal. The single bundle enters the free tepal (Fig. 5 B). Three of the five inner bundles split at the ovary top into dorsal carpel traces and traces of the outer three stamens. The remaining two bundles enter unchanged into the two inner stamens. The above mentioned 5 bundles differ from the perianth traces including that of the free tepal. Each of the vascular bundles which supply the stamens has the appearance of two collateral bundles fused tangentially in such a way that the xylem obtained a median position and the phloem groups are situated on two sides of the "fused" xylem (Fig. 11 B). Occasionally we have sectioned flowers with 6 stamens. In these cases such a vascular bundle appeared also opposite the third nectary lobe. Our observations thus support the first mentioned, and more generally accepted, interpretation of the banana flower, namely that the perianth is six-membered and that the adaxial stamen of the inner whorl was lost during evolution.

References

Alexandrovicz, L. 1955. Etude development de l'inflorescence du bananier nain. *I. F. A. C. Ann.* **9**, p. 35.

Baumgartner, P. 1913. Untersuchungen an Bananenblütenstaender. *Beih. bot. Zbl.* **30:** 237-268.

Fahn, A. 1949. Studies in the ecology of nectar secretion. *Palest. J. Bot. Jerusalem* **4:** 207-224.

——. 1953. The origin of the banana inflorescence. *Kew Bull.* **1953:** 299-306.

——, N. Klarman-Kislev, and D. Ziv. 1961. The abnormal flower and fruit of May-flowering Dwarf Cavendish bananas. *Bot. Gaz.* **123:** 116-125.

Ram, H.Y.M., M. Ram, and F.C. Steward. 1962. Growth and development of the banana plant. 3. A. The origin of the inflorescence and development of the flowers. B. The structure and development of the fruit. *Ann. Bot. N.S.* **26:** 657—673.

Simmonds, N. W. 1959. *Bananas*. Longmans, London.

Stoler, S. 1960. *The Banana*. In *Notes and Studies*, Hassadeh, Tel Aviv (in Hebrew). 75-165.

White, P. R. 1928. Studies on the banana. An investigation of floral morphology and cytology of certain types of the genus *Musa* L., *Zeitschr. Zellforsch. mikrosk. Anat.* **7:** 673-733.

Adv. Pl. Morph. 1972: 170-178

CENTRIFUGAL PRIMORDIAL INCEPTION IN FLORAL DEVELOPMENT

BY ROLF SATTLER

Biology Department, McGill University, Montreal, Canada

ABSTRACT

Primordia which may be initiated centrifugally are those of the sepals, members of the epicalyx, petals, members of the corona and disc, stamens, staminodes, ovules, and integuments. The primordia of one set of appendages may be *centrifugal to* those of another, more centrally formed, set of appendages. For example, the petals may be centrifugal to the stamens. If not all petals are initiated after the stamens, the corolla is only *partially centrifugal* to the androecium. Such partial centrifugal inception is known also with regard to the calyx. *Centrifugal inception within a set of primordia* occurs when the primordia of this set are arranged at more than one level and are formed from inside to outside. The innermost primordia of this set may be centripetal within the floral bud as a whole (e.g., in centrifugal androecia) or they may be centrifugal to more centrally located primordia of another set (e.g., the calyx in some Compositae).

In a floral bud one, two, or three kinds of appendages may be formed centrifugally. For example, in *Populus tremuloides* only the stamens are centrifugal. In *Coris monspeliensis* two kinds of appendages, outer sepals and petals, are centrifugal. Finally, in *Lythrum salicaria* three kinds of appendages are centrifugal, namely outer sepals, petals and inner stamens. If one considers ovules and integuments as appendages, five kinds of appendages are centrifugal in the latter species.

Some aspects of the significance of centrifugal inception for morphogenesis and systematics are mentioned.

INTRODUCTION

In the vegetative region, leaf primordia are formed in an acropetal sequence on the shoot apex. In the reproductive region, the same pattern occurs. However, in the floral buds of quite a large number of taxa basipetal (=centrifugal) modes of inception have been reported. In these cases some (or at least one) primordia are initiated after the inception of more centrally located primordia. This kind of sequence is best known for the androecium (see, e.g., Corner, 1946; Leins, 1964a, 1971, 1972; Cronquist, 1957, 1968). But it occurs also in other parts of the flower in a variety of patterns. The purpose of this paper

---

I am most grateful to Dr. Peter Leins for constructive criticism of the preliminary manuscript and a thorough discussion which have improved the final manuscript considerably. I also wish to thank Professor Hermann Merxmüller who read and commented upon the draft manuscript. Financially the work was supported by grant A 2594 of the National Research Council of Canada.

is to draw attention to the diversity of centrifugal modes of primordial inception, to survey all the modes that are known to-date, (but not all the taxa that exhibit it), and to mention some aspects of the significance of centrifugal inception for floral morphogenesis and systematics.

Modes Of Centrifugal Primordial Inception

Unless one considers a stamen fascicle as a male flower, no flower is known which is completely centrifugal in its development. Theoretically, one would obtain such a flower if one assumes loss of perianth and gynoecium in a flower with a centrifugal androecium. Although it is not impossible that one may find such a type of flower, or produce it, the flower that are known to-date are either completely centripetal in their development or show a combination of centripetal and centrifugal modes of primordial inception, i.e., on one flower some (or at least one) appendages are initiated centripetally and others centrifugally.

Before the various sequences of primordial inception are described, definitional precisions have to be made. "X is centrifugal to Y" means that the primordia of the set X are formed after and outside those of the set Y. For example, when all sepal primordia are initiated after all the petal primordia, they are centrifugal to the petal primordia. If they are initiated after the petal and stamen primordia, they are centrifugal to the petal and stamen primordia; and so forth. "X is formed, partially centrifugal to Y" means that not all members of X are formed after Y (which is inside X). For example, the corolla of *Pisum sativum* is partially centrifugal to the outer whorl of the androecium: the first two petal primordia (carina) are initiated after the first (abaxial) stamen primordium; but the last two petal primordia (alae) are formed before the last two outer stamen primordia. "Centrifugal inception within X" means that the sequence of inception in a set X of primordia (that are arranged at more than one level) is from inside to outside of the floral bud. The innermost members of X may be centrifugal to more centrally located primordia or they may be centripetal with regard to the floral bud as a whole. The best example of the latter case is a centrifugal androecium. It should be noted that "centrifugal androecium" refers to centrifugal origin within the androecium, although it does not exclude the theoretical possibility that, in addition, the androecium is centrifugal to the gynoecium. In contrast, "centrifugal corrolla" means that the corolla is centrifugal to the androecium and/or gynoecium. When the corolla consists only of one whorl of petals, as it is usually the case, "centrifugal inception within the corolla" is theoretically impossible. It is obvious that "centrifugal inception of X" or "centrifugal X" is ambiguous from a strictly definitional point of view, although it is usually clear whether it means "centrifugal inception within X" or "X is centrifugal to Y".

In the following, centrifugal inception is reviewed separately for the various appendages.

Calyx.—In many taxa in which the calyx is rudimentary we observe centrifugal sepal inception. For example, in *Anthriscus sylvestris* the extremely reduced calyx is formed centrifugally to the corolla, androecium and gynoecium (Sattler, 1972). However, contrifugal calyx inception is not restricted to such cases. The calyx of *Stylidium adnatum* ' which is well developed and typical is initiated centrifugally to the androecium (Sattler, 1972). In the Compositae where the calyx may be highly modified, centrifugal inception has also been observed (see, e.g., Scheeffer-Pomplitz, 1957; Sattler, 1972). The same is true for *Valeriana officinalis* (Sattler, 1972) and related taxa. When an epicalyx (outer calyx) is present, it may be initiated centrifugally to the calyx (inner calyx) as, for example, in *Fragaria vesca* (Sattler, 1972).

Since the calyx usually consists only of one whorl of appendages, centrifugal inception within the calyx is theoretically impossible. In the few cases of a calyx with more than one whorl of sepals both centripetal and centrifugal inception has been observed. For example, in *Tragopogon pratensis* the innermost pappus members arise first and the outermost last; thus, centrifugal inception occurs also within the calyx (although a calyx whose members need not be totally homologous with phyllomes). In this case the innermost calyx members are centrifugal to the corolla and androecium. If the epicalyx is considered as an outer whorl of calyx, then centrifugal epicalyx inception would be another instance of centrifugal inception within the calyx.

Corolla.—Since the growth rate of petal primordia usually is much slower than that of the stamen primordia, one cannot deduce centrifugal petal inception from the fact that the petal primordia are much smaller than the stamen primordia. Such a false conclusion may have been drawn in some reports on centrifugal petal inception. Nonetheless, in several cases centrifugal petal inception has been firmly established. For example, in the Primulaceae it has been shown by several authors that the petal primordia are initiated after the stamen primordia on the abaxial side of the latter (see Sattler, 1962, 1972). In *Anthriscus sylvestris* the petals arise on the young receptacle; they are probably only partially centrifugal to the androecium since the last two stamen primordia do not clearly precede inception of all petals (Sattler, 1972). In *Stylidium adnatum* the petal primordia are also formed on the young receptacle centrifugal to the androecium but centripetally to the calyx (Sattler, 1972).

Corona.—The corona is usually formed centrifugally to the androecium and/or gynoecium, even in cases like *Asclepias syriaca* where it is very prominent and assumes the function of a corolla (Puri and Shiam, 1966; Sattler, 1972).

Perianth.—Not many instances are known in which an undifferentiated perianth arises centrifugally. In grasses, e.g., *Hordeum vulgare*, the lodicules (which are usually considered as perianth homologues) are initiated centrifugally to the stamens (Sattler, 1972). In *Scirpus validus* the inner perianth is partially centrifugal to the androecium. One member of the outer perianth seems to be even centrifugal to the gynoecium whereas the other two outer tepals arise centripetally (Sattler, 1972). According to Barnard (1957) all of these tepals are formed centripetally.

Androecium.—A large number of taxa of Takhtajan's (1969) Dilleniidae and other taxa such as *Lagerstroemia, Punica, Limnocharis,* and *Hydrocleis* show centrifugal inception within the androecium (see, e.g., Corner, 1946; Leins, 1964a, 1971; Kaul, 1967, 1968; Mayr, 1969; Sattler, 1972). In some of these cases like *Paeonia* (Hiepko, 1964) or *Limnocharis* (Kaul, 1967) the stamen primordia arise centrifugally on the expanding receptacle. However, in the majority of cases the stamen primodia are initiated on androecial primordia (also called fascicle primordia) that have been formed in a centripetal sequence on the floral apex. Examples of such cases are *Hypericum* (Leins, 1964a; Sattler, 1972), *Lagerstroemia, Punica* (Mayr, 1969) and polyandric members of the Malvales (Heel, 1966; Sattler, 1972). Borderline cases between the two patterns occur, for example, in *Hibbertia scandens* (Sattler, 1972) where the androecial (fascicle) primordia are hardly noticeable before the inception of individual stamens (see also Hiepko, 1964). In the male floral buds of *Populus tremuloides* centrifugal stamen inception occurs in two directions; some of the stamen primordia are formed directly on the floral apex, others on an abaxial centrifugal androecial ridge, i.e., on a fascicle primordium (Sattler, 1972). Interprimordial growth may occur between or underneath fascicle primordia. This growth may be so pronounced that as the ratio of (fascicle) primordail and interprimordial growth becomes one, a circular bulge results on which the stamen primordia are formed centrifugally, as, e.g., in *Capparis* and *Helianthemum* (Leins, 1964b).

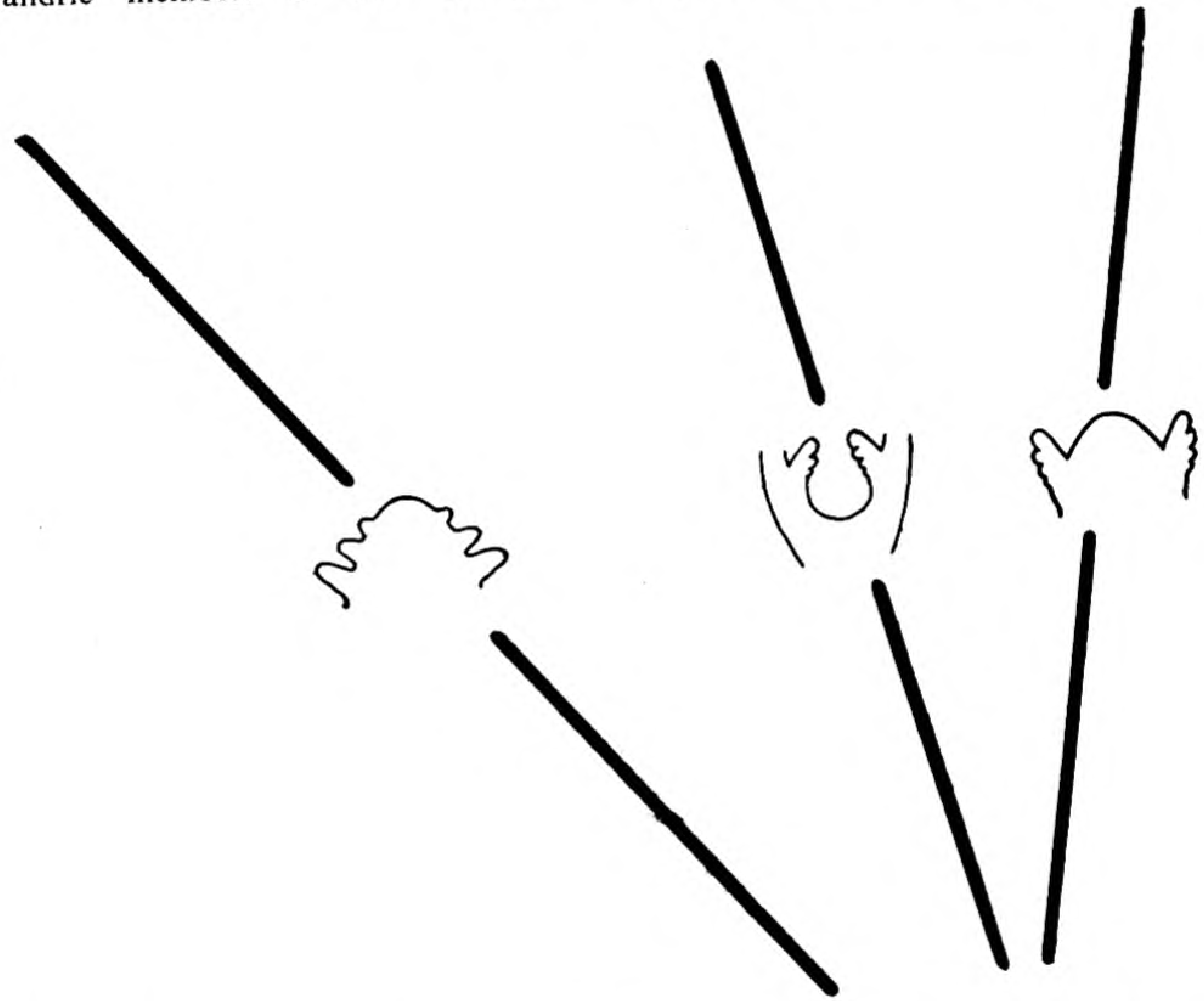
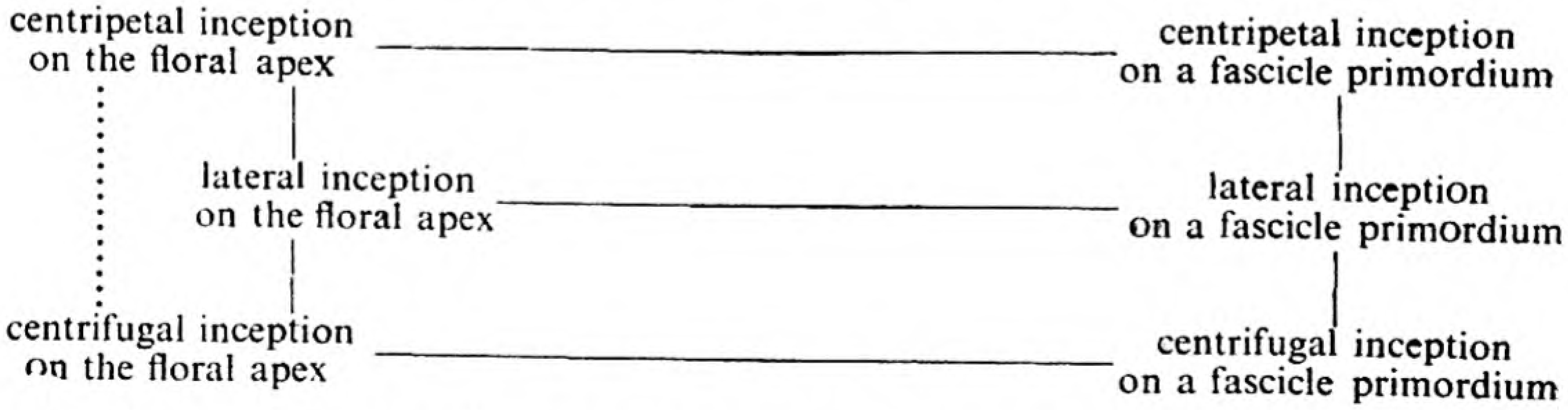


Fig. 1. Diagrammatic longitudinal sections through the distal portion of floral buds with centripetal (left and middle) and centrifugal (right) stamen inception. The centripetal mode of inception in the middle and the centrifugal mode to the right are considered to be more closely related according to Leins because in both cases the stamens are formed on fascicle primordia. The solid lines indicate evolutionary relationships (After Leins, 1971).

When stamen primordia are formed

on androecial (fascicle) primordia, their sequence of inception is meant to refer to the floral apex, but it could also refer to the apex of the fascicle primordium. In the latter case, both centripetal and cenrifugal sequences (with regards to the floral apex) are basipetal, i.e., centrifugal with regard to the fascicle apex (Fig. 1). In this sense, Leins (1964) compared fascicles of stamens with compound leaves, the primordia of the stamens being the equivalents of the leaflet primordia which appear in basipetal sequence. If one interprets stamen formation on fascicle primordia in this fashion, then the phenomenon of centrifugal stamens loses much of its peculiarity and becomes a special case of a phenomenon known in the vegetative region. However, one need not necessarily homologize a stamen fascicle with a compound leaf. It could also be homologized with a short shoot or it could be considered partially homologous with phyllomes and shoots (Heel, 1966). Even then, the reproductive and vegetative regions share the phenomenon of centrifugal inception, although the organs formed from the primordia may be only partially homologous with each other.

As long as stamen inception occurs on fascicle primordia, centrifugal and centripetal modes may gradually merge with each other as a mere result of positional change. In the case of centripetal stamen inception the stamen primordia are initiated on the ventral (adaxial) side of the fascicle primordium, whereas in the centrifugal condition they are formed on the dorsal (abaxial) side of the fascicle primordium. One can assume that the ventral position gradually changes into the dorsal position or vice versa, thereby transforming a centripetal androecium into a centrifugal one, or vice versa. The intermediate position is lateral. This is neither centrifugal, nor centripetal. Such a case of lateral stamen inception on a fascicle primordium has been actually reported for *Melaleuca nesophilla* (Leins, 1965) and *Aegle marmelos* (Leins, 1967). In the genus *Melaleuca* both lateral and ventral (centripetal) inception occurs (Mayr, 1969). Heel (1966) found transitional arrangements between dorsal (centrifugal) and lateral stamen formation in different genera of the Malvales. In some genera of the Malvales stamen formation on the fascicle starts centrifugally and becomes gradually centripetal (Heel, 1966). This shows that there is a wide range of variation and a morphological continuity between centripetal and centrifugal stamen inception as long as it occurs on fascicle primordia. In the following scheme morphological continuities (solid line) and discontinuities (dotted line) are summarized:


centripetal inception on the floral apex
centripetal inception on a fascicle primordium
lateral inception on the floral apex
lateral inception on a fascicle primordium
centrifugal inception on the floral apex
centrifugal inception on a fascicle primordium

One can see that in a polyandric androecium centripetal and centrifugal inception are discontinuous on the floral apex, i.e., they cannot merge with each other. However, if the androecium consists of two whorls, a gradual change of the centripetal pattern into the centrifugal one and vice versa is possible by positional change only. As the antepetalous stamens gradually become inserted outside the antesepalous stamens, the sequence of inception changes from centripetal to centrigfugal—and the androecium becomes obdiplostemonous. Eckert (1966) reported a range of transitional androecia between clearly diplostemonous and obdiplostemonous conditions. Some of the weakly obdiplostemonous androecia are due to centrifugal inception of the antepetalous stamens. Difficulties may arise because the margins of the primordia often cannot be clearly delimited and the centre of primordia is also not always easily determined. Furthermore, the situation may be different with regard to the inner and outer margins. For example, in *Silence cucubalus*, the inner margin of the second (antepetalous) staminal whorl is outside that of the first (antesepalous) staminal whorl. Consequently, inception is centrifugal with regard to the inner margin of the stamen primordia. But with regard to the outer margin of the same primordia the situation is reversed, i.e., inception is centripetal (Sattler, 1972).

In some cases stamens may arise centrifugally to the gynoecium. For example, in *Pisum sativum* the majority of stamens are centrifugal to the carpel (Sattler, 1972).

Like stamens, staminodia may also arise centrifugally. For example, in *Hydrocleis nymphoides* the last primordia which are formed in the centrifugal androecium develop into staminodia (Kaul, 1968). In *Mesembryanthemums* Ihlenfeldt (1960) found that even the petals are initiated within the centrifugal sequence of stamens and staminodes, i.e., androecium and corolla originate in one centrifugal sequence.

Disc.—As pointed out by Professor Puri (1951), the term 'disc' is not precisely defined and is used for a variety of structures. It is understood here in its widest sense including circular (disc-like) structures as well as whorls of small appendages which have been homologized with phyllomes or emergences. Discs of all sorts are usually formed centrifugally to the gynoecium. Examples are *Comandra umbellata, Fagopyrum sagittatum, Rhus typhina, Fuchsia hybrida, Calystegia sepium, Tagetes patula* (Sattler, 1972).

Gynoecium.—Centrifugal inception of carpel primordia within the gynoecium is not known, although it is theoretically possible in gynoecia with carpels at more than one level. Since ovules may be formed on the floral apex or tissue which is in continuation of the floral apex, the concept of "centrifugal primordial inception" may be applied to ovule inception, at least in cases of axile and free central placentation. Members of the Primulaceae, *Silene cucubalus, Solanum dulcamara*, etc., are examples of centrifugal (basipetal) ovule inception (Sattler, 1972).

With regard to the apex of the ovule one can apply the concept of "centrifugal inception" to the formation of integuments. In bitegmic ovules the outer integument is usually formed centrifugally to the inner integument (see, e.g., Kapil and Vasil, 1963; Puri, 1970). When an aril is present, it arises centrifugally to the integuments. The middle integument in some annonaceous plants (Corner, 1949, Periasamy and Swamy, 1961) is centrifugal to the inner integument and centripetal to the outer integument.

Usually only one kind of appendage is initiated centrifugally in one flower.

In some cases two kinds of appendages are initiated centrifugally in the same flower. For example, in the floral buds of *Coris monspeliensis* the outer calyx (epicalyx) is formed centrifugally to the inner calyx and the corolla centrifugally to the stamens (Sattler, 1962).

Lythrum salicaria so far is the only case in which three kinds of appendages are formed centrifugally (Cheung and Sattler, 1967; Sattler, 1972). In addition, the ovules and the outer integument arise centrifugally. If one includes "ovules" and "integument" in the category "appendage", five kinds of appendages are formed centrifugally in this species. Concerning the exposed appendages, the outer calyx (epicalyx) is centrifugal to the inner calyx, the inner whorl of stamens is centrifugal to the gynoecium, and the corolla is centrifugal to the gynoecium and androecium. The ovules arise centrifugally on the axile placentae. The outer integument is formed after the inner one. Thus, the sequence of primordial inception is the following: inner sepals, outer sepals, outer stamens, gynoecial wall, inner stamens, petals, ovules (inner integument, outer integument).

CENTRIFUGAL INCEPTION AND MORPHOGENESIS

We don't have an insight into the genetical and physiological mechanism which causes centrifugal primordial inception. But we begin to understand that centrifugal inception itself may be a cause for certain morphogenetic events during the further development of the flower. One such causal influence of centrifugal inception concerns the pattern of procambial differentiation. The procambial strand of a primordium usually differentiates from a strand(s) that is nearest to it. Since a change from a centripetal to centrifugal sequence may change the relative positions of primordia and procambial strands to each other, more or less different patterns of vascularisation may result. Thus, a change in the sequence of primordial inception, may be understood as a cause of changes in vascularisation. More important than sequential changes are positional changes in this respect. But sequential changes may be one factor among the net of factors that are responsible for the pattern of vascularisation.

CENTRIFUGAL INCEPTION AND SYSTEMATICS

Modern taxonomists agree that all available characters should be used for the construction of a natural system of classification. Consequently, the character of "sequence of primordial inception" with its states "centripetal" and "centrifugal" and "simultaneous" cannot be ignored. Since this character applies to all kinds of floral appendages (including ovules), it may assume some importance even in a system which is based on the philosophy of equal weighting of characters. The multitude of positional arrangements of the primordia provides additonal characters closely related to the sequential character.

In a taxonomic system of unequal weighting of characters the question of the importance of the sequence of primordial inception arises. No agreement has been reached in this respect. While most authors have given very little weight to sequential events, some have considered at least one of these events, namely stamen inception, as of great importance. Cronquist (1957) used this character to distinguish among the dicotyledons two major branches: a centrifugal and centripetal branch. In his later book (Cronquist, 1968) the basic idea of the importance of the sequence of stamen inception is retained, although the system of classification has been adapted to that of Takhtajan. It is very questionable whether such great importance should be attached to the sequence of stamen inception. Pauzé (personal communication) found that in *Ochna* (which belongs to Cronquist's centrifugal branch) the stamens are formed centripetally. This observation indicates that centrifugal stamen inception is no defining and diagnostic character state for a group of high taxonomic rank such as Cronquist's centrifugal branch. Also in the centripetal branch (or the centripetal groups) exceptions have been reported. Mayr (1969) found that within the Myrtales the Myrtaceae have a centripetal androecium, whereas *Lagerstroemia* (Lythraceae) and *Punica* (Punicaceae) show centrifugal inception within the androecium. Even at the family level we may find occasionally high plasticity with regard to the sequence of inception. In the Butomaceae both centrifugal and centripetal stamen inception occur (see, e.g., Kaul, 1967, 1968; Sattler, 1972). Hence, evolution of taxa with centripetal androecia into those with centrifugal androecia (and perhaps vice versa) is probably not such a fundamental step as envisaged by Cronquist, but a process which may have occurred several times: during the evolution of the Butomaceae, the Myrtales, perhaps in the Fagales (see Macdonald, 1971) and at least once during the evolution of the Dilleniidae, i.e., Cronquist's centrifugal branch. Similarly, the centrifugal inception of other appendages has evolved independently a number of times with great probability. For example, a centrifugal corolla has evolved independently in the Primulales, the Apiaceae, and the Stylidiaceae. A centrifugal calyx has evolved independently even more often.

It should be emphasized, however, that sequences of primordial inception are constant in many taxa of different ranks and may be used, in these cases, as defining or diagnostic characters. For example, at the ordinal rank we find centrifugal stamen inception in all (polyandric) members of the Malvales that have been investigated so far (Heel, 1966), although in some genera it is partially centripetal. At the family level centrifugal petal inception characterizes the Primulaceae. Similarly, all members of the Primulaceae exhibit centrifugal ovule inception. Other examples of taxa of lower rank, including the species level, could be added (see Sattler, 1962).

Constancy of sequential patterns as well as their variation has been utilized to support phylogenetic speculations. For example, the occurrence of a centrifugal corolla in all members of the Primulaceae, together with other evidence, has been considered as a confirmation of monophyletic origin. The fact that in the Theophrastaceae and Myrsinaceae genera with a centrifugal corolla are known in addition to genera which link the centrifugal corolla with the centripetal corolla (Sattler, 1967), has been used to support the phylogenetic derivation of the Primulaceae from ancestors related to the Theophrastaceae and Myrsinaceae (Sattler, 1962). It is needless to say that such inferences are not made on the sole ground of sequential events. Sequential events are used in conjunction with other data. As Professsor Puri has pointed out repeatedly, there is no single aspect of enquiry that is the only "way to salvation".

REFERENCES

Barnard, C. 1957. Floral histogenesis in the Monocotyledons. II, The Cyperaceae. *Aust. J. Bot.* **5:** 115-128.

Cheung Matilda, and R. Sattler. 1967. Early floral development of *Lythrum salicaria*. *Can. J. Bot.* **45:** 1609-1618.

Corner, E. J. H. 1946. Centrifugal stamens. *J. Arnold Arb.* **27:** 423-437.

——. 1949. The annonaceous seed and its four integuments. *New Phytol.* **48:** 332-364.

Cronquist, A. 1957. Outline of a new system of families and orders of dicotyledons. *Bull. Jard. Bot. Bruxelles* **27:** 13-40.

——. 1968. *The Evolution and Classification of Flowering Plants*. Boston, Houghton Mifflin Co.

Eckert, Gertrud. 1966. Entwicklungsgeschichtliche und blütenanatomische Untersuchungen zum Problem der Obdiplostemonie. *Bot. Jahrb.* **85:** 523-604.

Heel, W. A. van. 1966. Morphology of the androecium in Malvales. *Blumea* **13**: 178-394.

Hiepko, P. 1964. Das zentrifugale Androeceum der Paeoniaceae. *Ber. Deutsch. Bot. Ges.* **77:** 427-435.

Ihlenfeldt, H. D. 1960. Entwicklungsgeschichtliche, morphologische und systematische Untersuchungen an Mesembryanthemen. *Feddes Rep.* **63:** 1-104.

Kapil, R. N. and I. K. Vasil. 1963. Ovule. In: *Advances in the Embryology of Angiosperms.* P. Maheshwari (ed.) Intern. Soc. Pl. Morphologist. Univ. Delhi, Delhi.

Kaul, R. B. 1967. Ontogeny and anatomy of the flower of *Limnocharis flava* (Butomaceae.) *Am. J. Bot.* **54:** 1223-1230.

——. 1968. Floral development and vasculature in *Hydrocleis nymphoides* (Butomaceae.) *Am. J. Bot.* **55:** 236-242.

Leins, P. 1964a. Die frühe Blütenentwicklung von *Hypericum hookerianum* Wight et Arn. und *H. aegypticum* L. *Ber. Deutsch. Bot. Ges.* **77:** 112-123.

——. 1964b. Das zentrifugale und zentripetale Androeceum. *Ber. Deutsch. Bot. Ges.* **77:** 22-26.

——. 1965. Die Infloreszenz und frühe Blütenentwicklung von *Melaleuca nesophila* F. Muell. (Myrtaceae) *Planta* **65:** 195-204.

——. 1967. Die frühe Blütenentwicklung von *Aegle marmelos* (Rutaceae). *Ber. Deutsch. Bot. Ges.* **80:** 320-325.

——. 1971. Das Androeceum der Dikotylen. *Ber. Deutsch. Bot. Ges.* **84:** 191-193.

——. 1972. Das zentrifugale Androeceum von *Couroupita guanensis*. *Beitr. Biol. Pfl.* (in press).

Macdonald, A. D. 1971. *Floral Development in Some Members of the "Amentiferae"*. Ph. D. Thesis. McGill Univ.

Mayr, Barbara. 1969. Ontogenetische Studien an Myrtales-Blüten. *Bot. Jahrb.* **89:** 210-271.

Periasamy, K, and B. G. L. Swamy. 1961. Studies in the Annonaceae. II. The development of ovule and seed in *Cananga odorata* and *Milisua wightiana*. *J. Indian bot. Soc.* **40:** 206-216.

Puri, V. 1951. The role of floral anatomy in the solution of morphological problems. *Bot. Rev.* **17:** 471-553.

——. 1970. The angiosperm ovule. *Proc. 57th Session Indian Sci. Congr.*, 1-36.

Puri, V. and R. Shiam. 1966. Studies in floral anatomy. VIII. Vascular anatomy of the flower of certain species of the Asclepiadaceae with special reference to the corona. *Agra Univ. J. Res. (Science)* **15:** 189-216.

Sattler, R. 1962. Zur frühen Infloreszenz- und Blutenentwicklung der Primulales sensu lato mit besonderer Berücksichtigung der Stamen-Petalum-Entwicklung. *Bot. Jahrb.* **81:** 358-396.

——. 1967. Petal inception and the problem of pattern detection. *J. Theoret. Biol.* **17:** 31-39.

——. 1972. *Organogenesis of flowers. A photographic text-atlas.* Toronto: Univ. of Toronto Press.

Scheefer-Pomplitz, and Marie-Edith. 1957. Morphologische Untersuchungen über den Pappus der Kompositen. *Beitr. Biol. Pfl.* **33:** 127-148.

Takhtajan, A. 1969. *Flowering Plants : Origin and Dispersal.* Translated by Jaffery Edinburgh: Oliver and Byod.

Adv. Pl. Morph. 1972: 179-192

AUTOTETRAPLOID OF *RAUVOLFIA SERPENTINA*

BY V. R. DNYANSAGAR AND S. G. TORNE

*Botany Department, Nagpur University, Nagpur-*10 *and S. P. Chowgule College, Madgaon, Goa, India*

ABSTRACT

Tetraploidy was induced in *Rauvolfia serpentina* (L.) Benth. ex Kurz by treating seeds with 0.5% of aqueous colchicine. Three naturally occurring tetraploids were also obtained while screening the population of diploid plants. The coefficient of variation of growth-rate is greater in the tetraploid than in the diploid. Root-yield is more in the tetraploid. The average total alkaloid content has been found to be 1.81% in diploid roots while it is 2.0% in the tetraploid ones. Reserpine content has not been observed to have increased much in the tetraploid roots, the value being more or less same (0.11%) as in the diploid roots.

Mitosis in the tetraploid is almost normal except in a few cells where grouping of chromosomes into masses at metaphase has been observed. Meiosis in both the naturally occurring and induced tetraploid is highly irregular. Some of the common irregularities are the presence of univalents, micronuclei, spindle abnormalities, irregular anaphase and formation of polyads. The phenomenon of cytomixis is exhibited by a few PMCs.

Correlation coefficients between bivalents and quadrivalents of the induced and natural tetraploids have been determined. There is significant negative correlation in both the cases. The cytological data indicate that no correlation exists between the univalent frequency and seed fertility or between the multivalent frequency and seed fertility.

INTRODUCTION

The drug reserpine from the roots of *Rauvolfia serpentina* (L.) Benth. ex Kurz belonging to the family Apocynaceae, has within the last few years gained prominence not only in India but all over the world in treatment of hypertension and insanity. As no detailed cytogenetic work was done on this medicinally important plant, the authors had undertaken such a study. Papers on the karyotype (Dnyansagar and Torne, 1967) and meiosis (Dnyansagar and Torne, 1969) of the diploid plant have already been published. The present paper deals with the cytogenetic study of the natural and induced tetraploid.

MATERIAL AND METHODS

Diploid plants of *R. serpentina* were raised from seeds and they were grown at the Botanical Garden, Institute of Science, Bombay. Healthy seeds were selected from such plants for the experimental work.

Sterilized seeds after rupturing on one

The authors express their thanks to the Maharashtra State Industrial Research Committee for the grant-in-aid under which the present work was carried out.

side to facilitate water absorption were first soaked for 48 hours and then treated for 24 hours with 0.25% and 0.5% aqueous colchicine solution.

Sterilized seeds after rupturing on one side were allowed to germinate in earthen pots. When the first pair of leaves opened, a small cotton plug previously soaked in 0.5% colchicine solution was placed on the terminal búd covered by an empty inverted pot so as to maintain sufficient humidity in the air surrounding the seedling. After 10 hours the apical buds were washed with water. After 7 days all the seedlings were treated with the above mentioned concentration for 10 hours.

Both 0.25% and 0.5% aqueous colchicine solutions were effective in inducing tetraploidy in *R. serpentina*. However, best results were obtained from seedlings treated for 10 hours with 0.5% colchicine solution.

Three naturally occurring tetraploids were also obtained in the garden while screening the population of diploid plants. In order to ascertain whether the naturally occurring tetraploids were auto- or allotetraploids, qualitative analysis of amino acids in the leaves of diploid, induced and natural tetraploid plants was carried out by using the paper chromatographic method.

Leaf smears were used for counting chromosomes. The material was fixed in Carnoys' fluid and acetocarmine was used for staining. Those seedlings where chromosome numbers were found to be $2n = 44$, were confirmed as tetraploids. Other criteria found useful for scoring tetraploids were (1) large size of the cotyledons and (2) lobing, bifurcation and fusion of the first formed leaves.

To measure the yield of root per plant, the former were carefully harvested. After obtaining the fresh weight of the harvested roots (50 plants in 5 groups), they were sun-dried. Assaying of the total alkaloid content from the powder roots was carried out following the procedures of Hamied and Bakshi (1956). Chromatographic papers were, however, run before spotting with pH 8.6 borate buffer.

For meiotic analysis the floral buds of the tetraploids were fixed for 48 hours in 4:3:1 chloroform-alcohol-propionic acid mixture. A small amount of $Fe(OH)_2$ was dissolved in propionic acid before a fresh mixture was prepared. The morphology of pollen grains was studied by employing the methods of acetolysis (Erdtman, 1952; Nair, 1962).

OBSERVATIONS

Morphological characters.—Many of the induced tetraploid plants did not show characteristic giganticism in their height and other morphological parts except in a few cases.

Height of diploid and induced tetraploid plants was measured every fortnight (Fig. 1). In the diploid plants the rate of growth was higher than in the tetraploids in the first and second fortnight. In the third and fourth fortnight the rate was not much pronounced while during the fifth and sixth fortnight, a distinctive rise was noticed. The coefficient of variation of growth-rate was found to be greater in the tetraploid than in the diploid (Fig. 2). Comparative data of morphological characters of diploid and tetraploid dlants (Fig. 3) are given in table I.

Fresh and dry weight of roots.—The mean height of the plant and fresh and air-dry weight percentages have been given in Table 2. In general roots of the tetraploids have been found to be slightly thicker and more branched (Fig. 5). It will be seen from table II that the variation in the root-yield of the tetraploid plants is distinctly more than that in the diploid plants of the same age (3 year growth). The maximum amount of fresh weight obtained from the roots of the tetraploid plant was 56.7 g. and the minimum was 50.9 g. The variation in the diploid was 41.8 g. to 36.8 g. per plant. Tetraploid roots contained less moisture percentage as compared with the diploid roots.

TABLE I

COMPARISON OF CHARACTERS OF DIPLOID AND TETRAPLOID *R. SERPENTINA*

Characters	Sample size	Mean±S.E. Diploid	Mean±S.E. Tetraploid	Value of t
Stem characters				
(a) Height of the plant in inches	13	32.5±1.70	23.6±0.82	2.379*
(b) Thickness of the stem at the first node in cm.	13	3.8±0.40	4.2±0.71	0.54
Leaf characters				
(a) Thickness of leaf in µ	13	387.5±3.2	735.5±3.8	3.2**
(b) Length of the palisade in µ	13	90.0±2.3	177 ±2.54	3.31**
(c) Breadth of the palisade in µ	13	17.6±0.8	28.9±0.76	2.1*
(d) Average number of epidermal cells (lower) per sq. mm.	13	743 ±4.6	358 ±3.96	2.38*
(e) No. of stomata per sq. mm.	13	234 ±1.3	123 ±1.02	2.26*
(f) Length of the stomatal aperture in µ	13	18.3±0.70	22.6±0.76	1.91
Floral characters				
(a) Length of the infl. axis in cm.	13	9.1±0.43	5.5±0.48	1.89
(b) Number of flowers formed on infl. axis.	13	112 ±2.2	36. ±1.52	4.3**
(c) Pollen fertility (per cent)		95.7±2.4	91.9±2.3	2.1*
(d) Pollen diameter in µ (Polar view), tricolporate grain		82.3±2.1	101 ±1.96	2.36*
Seeds				
(a) No. of seeds formed (%)		48 ±2.2	12 ±1.46	3.36**

* Significant at 0.05 % level; ** Significant at 0.01 % level.

Estimation of total alkaloid content and reserpine.—The average total alkaloid was found to be 1.81 % in diploid roots

TABLE II

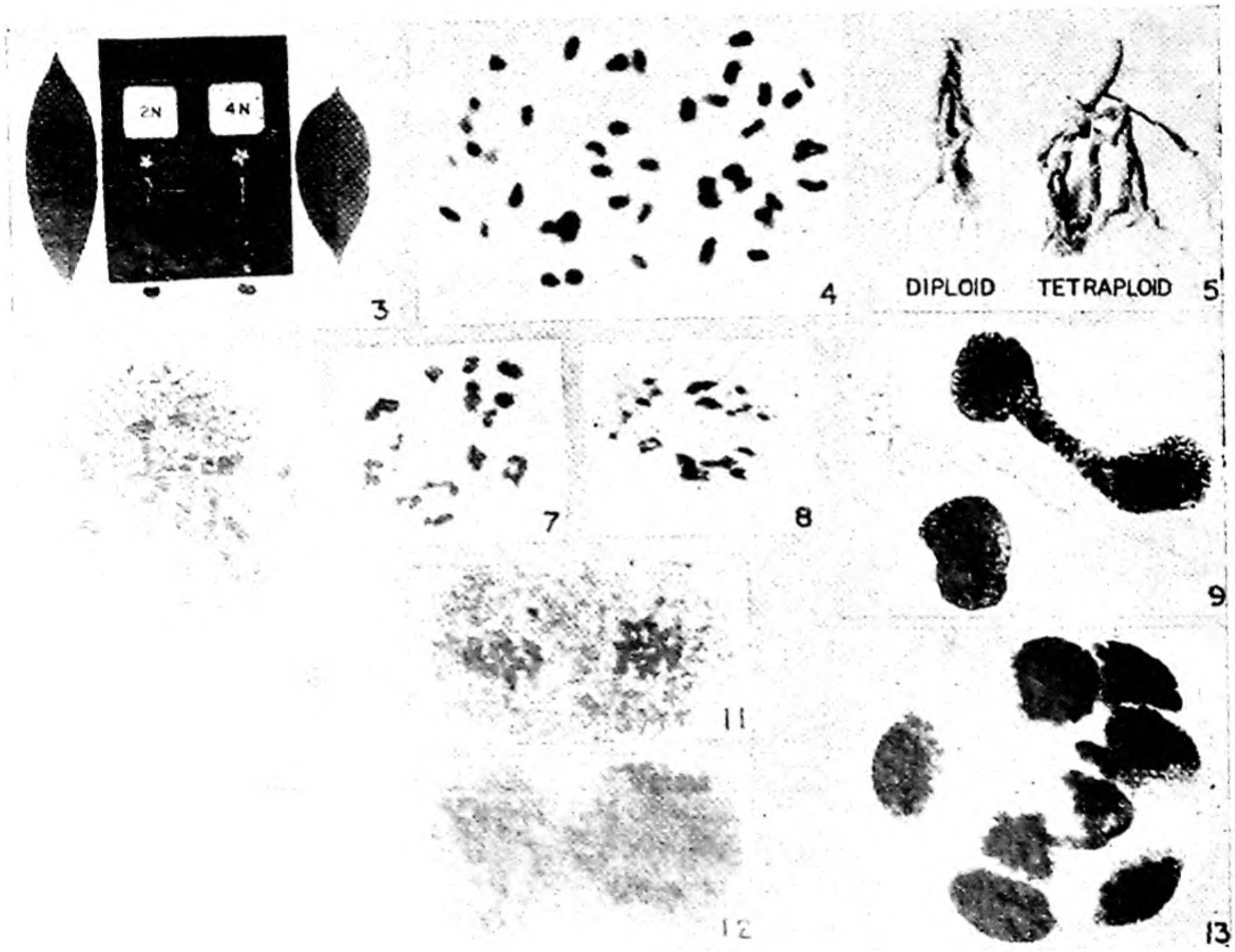
MEAN HEIGHT OF THE PLANT, FRESH WEIGHT AND DRY WEIGHT OF ROOT PERCENTAGE OF DIPLOID AND TETRAPLOID PLANTS OF *R. SERPENTINA*

Characters	Diploid No. of plants 1	2	3	4	5	Mean	Tetraploid No. of plants 1	2	3	4	5	Mean	4n/2n
Height in inches	35.7	32.3	42.1	29.7	31.8	34.32	23.6	21.7	22.7	20.2	24.5	22.54	0.65
Fresh weight in g.	40.8	36.8	37.9	41.8	40.1	39.48	52.6	55.3	53.6	56.7	50.9	53.62	1.35
Air dried percentage	20.3	19.5	17.4	20.5	19.6	19.46	30.7	31.5	31.5	32.6	33.3	33.2	1.64

while it was 2.01 % in tetraploid roots i.e. there was an increase of 11% of total alkaloid in the tetraploid roots. Reserpine content was not found to have increased much in the tetraploid roots, the value being more or less same as in the diploid roots where it was 0.11%.

Mitosis and karyotype.—The process of mitosis was found to be almost normal except in a few cells where grouping of chromosomes into masses at metaphase was observed.

The karyotype analysis of both the natural and induced tetraploid plants



[illegible] Mean rate of growth of 2n and 4n plants. Fig. 2. Coefficient [illegible] points. Fig. 3. Comparative morphological characters of diploid and [illegible] chromosomes from a root-tip cell showing 2n=44, x 2000. Fig. 5. Roots of dip[illegible] PMC showing 1 pentavalent, 7 tetravalents, 1 trivalent and 4 bivalents, x 1345. [illegible] trivalent, 6 tetravalents, 6 bivalents and 2 univalents, x 1345. Fig. 8. PMC showing [illegible] bivalents, x 1345. Fig. 9. PMCs showing cytomixis between two, x 1000. [illegible] cytomixis between 3 PMCs, x 1000. Fig. 11. PMC showing the precocious movement of [illegible], x 1300. Fig. 12. Laggards at anaphase II, x 1300. Fig. 13. Formation of polyad, x 1300.

indicates that it is similar to that of the diploid plants (Dnyansagar and Torne, 1967). The chromosome number was found to be 44 (Fig. 4). The length of chromosomes varies from 3.98 μ to 1.21 μ. Two pairs of SAT-chromosomes are found in the compliment. Tetraploids are composed of 4 complements of similar chromosomes indicating that the tetraploids (induced and natural) of *R. serpentina* are autotetraploids.

TABLE III

CHIASMA FREQUENCY AT METAPHASE I IN THE INDUCED AND NATURAL TETRAPLOIDS OF *R. SERPENTINA*

	No. of PMCs	No. of configurations with chiasmata					Total Chiasmata	Chiasmata per cell	Chiasmata per bivalent	Coefficient of terminalization.
		0	1	2	3	4				
Induced tetraploid	15	6	11	110	4	77	551	36.74	1.67	0.43
Natural tetraploid	15	8	12	120	2	65	508	33.86	1.54	0.5

Meiosis.—Efforts to homologize chromosome behaviour and stages of meiosis in the present material encountered many difficulties. The interpretation of meiosis in the material became difficult due to irregularities and absence of a uniform and regular sequence of events and wide ranges of stages found randomly distributed among the PMCs in a single anther. The nuclear upset was manifested even during prophase I in some PMCs. Densely staining chromocentres precluding usual chromosome behaviour and resulting in death of such cells were also observed. In some PMCs which had not completely arrested such disturbances, caused stickiness with one another. Precocious separation of chromosomes sometimes occurred at or before diakinesis.

(a) *Diakinesis* (Figs. 6-8).—At diakinesis the chromosomes were slightly spread but just at prometaphase, in a few cases, they formed compact rounded balls or irregular configurations.

Configurations observed at diakinesis were chains and rings of two and four chromosomes, respectively while trivalents appeared only as chains. Univalents never exceeding two per PMC, were observed in 0.60% of the cells in the induced tetraploid and they invariably accompanied trivalents. The most common observation was the chain or ring of two chromosomes while open and closed quadrivalents were seen in 4.93% of the cells in the induced tetraploid and their maximum number in any PMC never exceeded 8. In the natural tetraploid the quadrivalent frequency was found to be 3.92. More quadrivalents were seen in the induced tetraploid rather than in the natural one.

Accompanying the variability of chromosomes was the variability in the number of chiasmata in the natural tetraploid as against in the induced tetraploid. In all plants (natural and induced) studied chaisma frequency in any of the tetraploid never equalled twice that of the diploid from which they were derived. The chiasma frequency and terminalization coefficient were found to be 1.67 and 0.43 respectively in the induced tetraploid and for the naturally occurring tetraploid the figures were 1.54 and 0.5, respectively (Table III). Lagging of chromosomes at metaphase I was observed which resulted in a delay in congression of the chromosomes at the metaphase plate.

Some of the common abnormalities seen were (1) poor development of spindle, (2) complete absence of bivalent formation in PMCs, (3) occurrence of tri- and tetravalents, and univalents invariably accompanying trivalents, (4) non-orinetation of most of the univalents and some of these aligning themselves on the metaphase plate, and (5) clumping of chromosomes on the equatorial plate. A common feature at the metaphase I was that chromosomes were oriented at two or more equatorial plates. These plates represented separate spindles and functioned independently during later stages of meiosis. The greatest difference in timing was observed in a cell with one group of chromosomes at late prophase I and the other at early metaphase I. Here the two groups were unequal. Two metaphase plates were parallel, and adjacent or distant but mostly at right angles to each other. This abnormality involved cytoplasmic irregularity and consequent failure of cytokinesis resulting in a mixing of some chromosomes of one plate with those of other. An occasional tripolar spindle and rarely a quadripolar spindle were also observed. In this tripolar configuration a group of quadri-, tri-, bivalents and some univalents were present at each of three equators.

Correlation coefficients between bivalents and quadrivalents of the induced and natural tetraploids were calculated. Significant negative correlation was observed in both the cases. The value for the correlation coefficients was -0.7840 for the induced tetraploid while for the natural tetraploid it was -0.9708. More quadrivalents than bivalents were observed in the induced tetraploid while in the natural tetraploid more bivalents than quadrivalents were present. An examination of the data presented in table IV indicates that the pronounced variation exists between the plants in their chromosome pairing associations. The means for different association in two generations tested have been found to be rather close in their numerical magnitude and the differences between the C_1 and C_2 generations are not significant.

(b) *Metaphase I.*—At this stage plate with several irregularly oriented chromosomes, was sometimes observed with other members of the compliment scattered throughout the cell. Some chromosomes were already at the poles while others were just becoming oriented at the plate. In other PMCs precocious and faulty spindle formation resulted in a complete absence of orientation of chromosomes.

(c) *Anaphase I.*—This was highly irregular with about 83.91% cells showing very interesting abnormalities viz., absence of an active polar movement, formation of a cell wall across the metaphase plate which could be seen persisting even after the completion of the cell wall and formation of dyads, first division restitution nucleus, and variable number of laggards. Although 87% cells had shown laggards at anaphase I, only 63% had micronuclei at the tetrad stage. In 3-5% cells bridges were observed. Sometimes univalents lagged on the spindle and like multivalents underwent a delayed separation. The presence of such univalents affected a spindle elongation and cell cleavages resulting in the formation of a restitution nucleus. At late anaphase I, a group of chromosomes at one pole separated into two groups which were lying somewhat apart. The separated groups were very much unequal in size.

(d) *Prophase II.*—At this stage chromosomes were usually clumped and two to several nucleoli of varying sizes were present. Often nucleolar-like material on the surface connected various chromosomes. The surrounding nuclear membrane was not visible and lagging chromosomes formed micronuclei. The configuration representing separation of one nucleus into two or fusion of two nuclei into one was also observed in prophase. Typically microsporocytes contained two macronuclei and frequently one or more separate micronuclei.

(e) *Metaphase II.*—The number of chromosomes at metaphase II had shown random separation varying from equal to very unequal. PMCs with precocious movement of a bivalent were also observed (Fig. 11). Several spindles at metaphase II gave rise to several chromosomal groups.

(f) *Anaphase II.*—In some cells very loose grouping of chromosomes was seen but they were not sharply delimited. The failure of plate formation and polar movement indicated a complete breakdown of the mechanism of cell division. The following abnormalities were mostly observed : (1) Subdivision of the chromosomes within the cell, (2) non-disjunction of chromosomes, (3) unequal distribution of chromosomes to the two polar regions and (4) bridges, strays and laggards (Fig. 12).

(g) *Telophase II.*—During this stage micronuclei were produced due to all aforesaid irregularities. There was also frequent occurrence of polyads (Fig. 13). The phenomenon of cytomixis was also exhibited by a few PMCs. The transfer

TABLE IV

CHROMOSOME ASSOCIATIONS AT METAPHASE I IN THE AUTOTETRAPLOIDS OF *R. SERPENTINA*

Plant studied	Chromosome associations						No. of cells analysed.
	I	II	III	IV	V	VI	
C_1 generation							
1	0.60	9.56	0.35	4.88	0.20	0.44	22
2	0.58	9.50	0.31	4.98	0.22	0.40	29
3	0.62	9.55	0.34	4.92	0.20	0.36	21
4	0.60	9 51	0.32	4.94	0.18	0.40	17
Mean	0.60	9.53	0.33	4.93	0.20	0.40	Total 89
C_2 generation							
1	0.59	9.57	0.29	4.92	0.20	0.36	26
2	0.65	9.53	0.33	4.96	0.16	0.40	23
3	0.64	9.51	0.32	4.95	0.19	0.39	25
4	0.60	9.59	0.30	4.93	0.17	0.37	28
Mean	0.62	9.55	0.31	4.94	0.18	0.38	Total 102

of chromatin material was between two (Fig. 9) or more cells (Fig 10).

Data on percentage of meiotic irregularities have been given in table V. Highly significant differences between individual plants are seen. There appear to be no significant differences between divisions and in the average percentages of abnormal PMCs. The coefficient of correlation between frequencies of abnormalities at various stages of divisions has been calculated and given in table VI. The correlation coefficients are also calculated between percentages of aborted pollen and the frequencies of univalents, quadrivalents, polyads, percentage of PMCs with univalents and of abnormal tetrads (r values 0.309, −0.165, −0.101; −0.012, 0.023, respectively) for five tetraploids of C_1 generation.

Ten plants of C_1 and C_2 generations were available for analysing the relationship between seed production and regularity of meiosis. The number of seeds per head is correlated with various data regarding frequencies of chromosome associations and irregularities during stages of meiosis. At the same time, the seed set appears to be correlated with pollen fertility (Table V).

TABLE V

PERCENTAGE OF MEIOTIC IRREGULARITIES, POLLEN ABORTION AND NUMBER OF SEEDS.

Plant and generation	Metaphase I % PMCs with univalents	% of abnormal PMCs Div. I	Div. II	Tetrads	Mean No. of polyads	% of abnormal pollen grains	No. of seeds.
C_1 generation							
1	28.0	33.3	28.7	33.9	0.66	11.7	14
2	25.7	38.5	23.0	22.1	0.44	13.5	8
3	42.1	41.9	39.4	35.6	0.69	11.3	16
4	48.9	56.0	54.7	32.0	0.49	14.7	10
Mean	36.17	42.4	36.6	30.87	0.56	12.8	12
C_2 generation							
1	33.7	82.6	35.6	31.2	0.53	12.7	12
2	41.6	35.4	37.5	24.4	0.59	12.9	10
3	42.5	33.6	42.6	26.6	0.55	13.2	8
4	31.5	44.5	41.3	36.3	0.55	14.2	6
5	29.2	51.9	35.5	39.5	0.63	13.5	9
Mean	35.7	39.6	38.5	31.63	0.57	13.3	9

DISCUSSION

Treatment.—*R. serpentina* being herbaceous responded well to 0.5% colchicine solution. Solutions of higher concentrations than 0.5% colchicine did not give satisfactory results and proved toxic at times. Janaki Ammal (1962) claimed to have obtained tetraploid *R. serpentina* plants with the treatment of colchicine but she did not, however, mention its concentration. Furusato (1940) by using 0.02% to 0.8% colchicine solution was successful in inducing tetraploidy in 13 diploid species of which one was *Vinca rosea* (*Catharanthus roseus*). Eigsti and Schnell (1943) reported that 1% solution of colchicine-in-glycerine proved more effective for the induction of polyploidy in *Vinca*. They obtained 5% of tetraploids out of 1000 diploids treated by brush method.

Soaking of seeds of *R. serpentina* with water for two days gave better results in the induction of polyploidy than dry seeds. The best result was obtained with 0.5% colchicine rather than with 0.25%. Koltzoff (1939) soaked *Vicia sativa* seeds for 1 to 12 days before subjecting them to colchicine and claimed better results than by treating unsoaked seeds.

Growth.—Arrested growth of the shoot of *R. serpentina* was the immediate effect observed as a result of the treatment with colchicine solution. Cotyledons and first leaves became thicker. Hawkes (1942) observed that the effect of colchicine on the root and cotyledons was the increase in their thickness with no corresponding increase in their length. Levan (1938) considered this subterminal root swelling to be due to the increase in cell volume consequent upon the larger number of chromosomes present after colchicine doubling. Hawkes (1942), however, did not agree with Levan. According to the former, the cells in the swollen zone were maturing ones and would have the ordinary diploid chromosome numbers. Consequently the size (volume) should not be greater than in the untreated material unlesss colchicine exerts an influence on the cell volume other than by increasing the number of chromosomes. He attributed the swelling to be due to destruction of polarity of cells under the action of colchicine as a result of which the cells which normally increased in length parallel to the root axis, increased in all dimensions.

TABLE VI

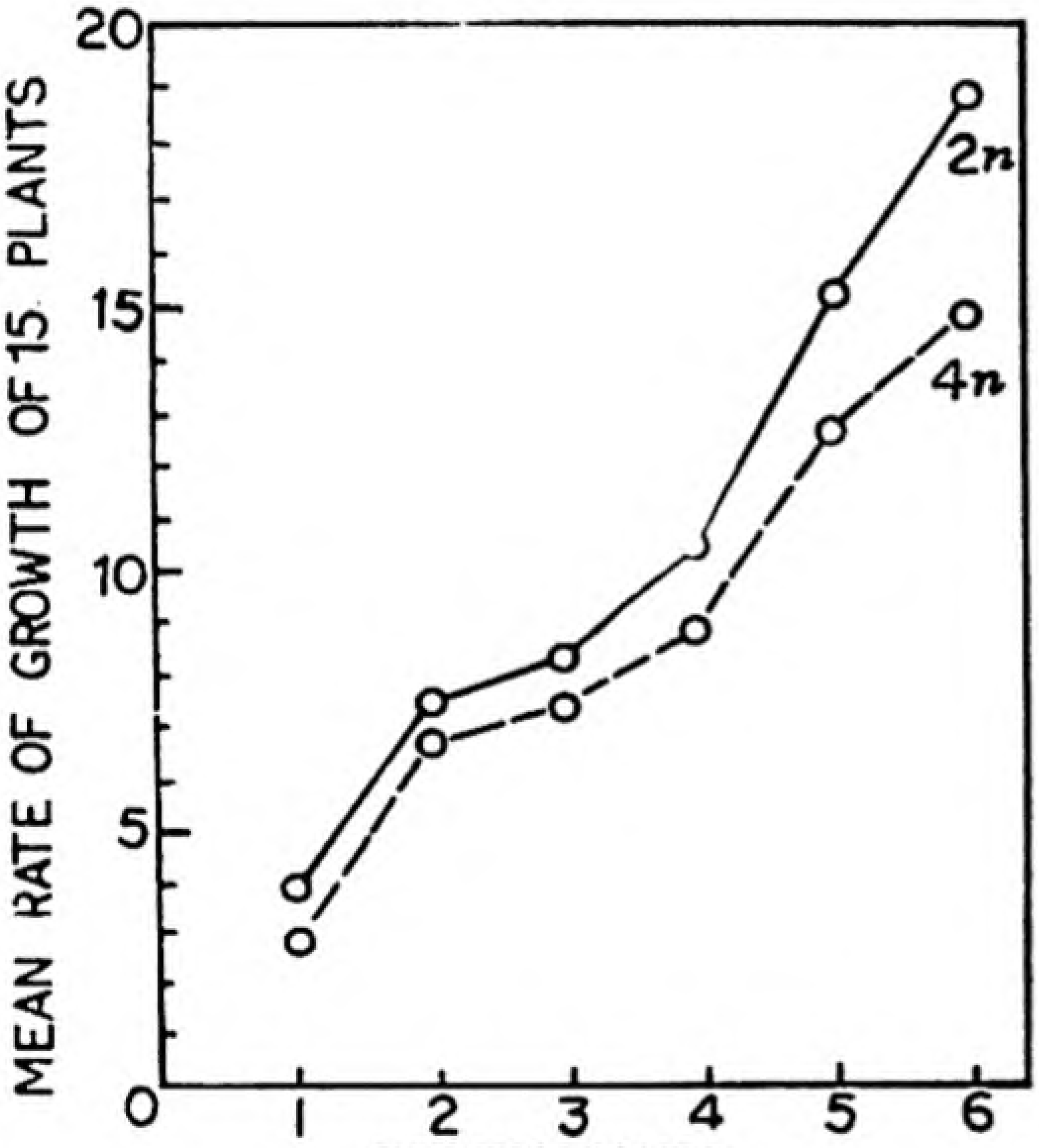
CORRELATION COEFFICIENTS BETWEEN ABNORMALITIES AT VARIOUS STAGES OF MEIOSIS IN 18 AUTOTETRAPLOIDS

	r
Univalents at M I and abnormalities at division I (Anaphase I and Telophase I)	0.559*
Univalents at M I and abnormalities at division II (Anaphase II and Telophase II)	0.844*
Univalents at M I and abnormalities at tetrad stage	0.782*
Abnormalities at division I and abnormalities at division II (Anaphase-Telophase)	0.479*
Abnormalities at division I and abnormalities at tetrad stage	0.346*
Abnormalities at division II and abnormalities at tetrad stage	0.720*

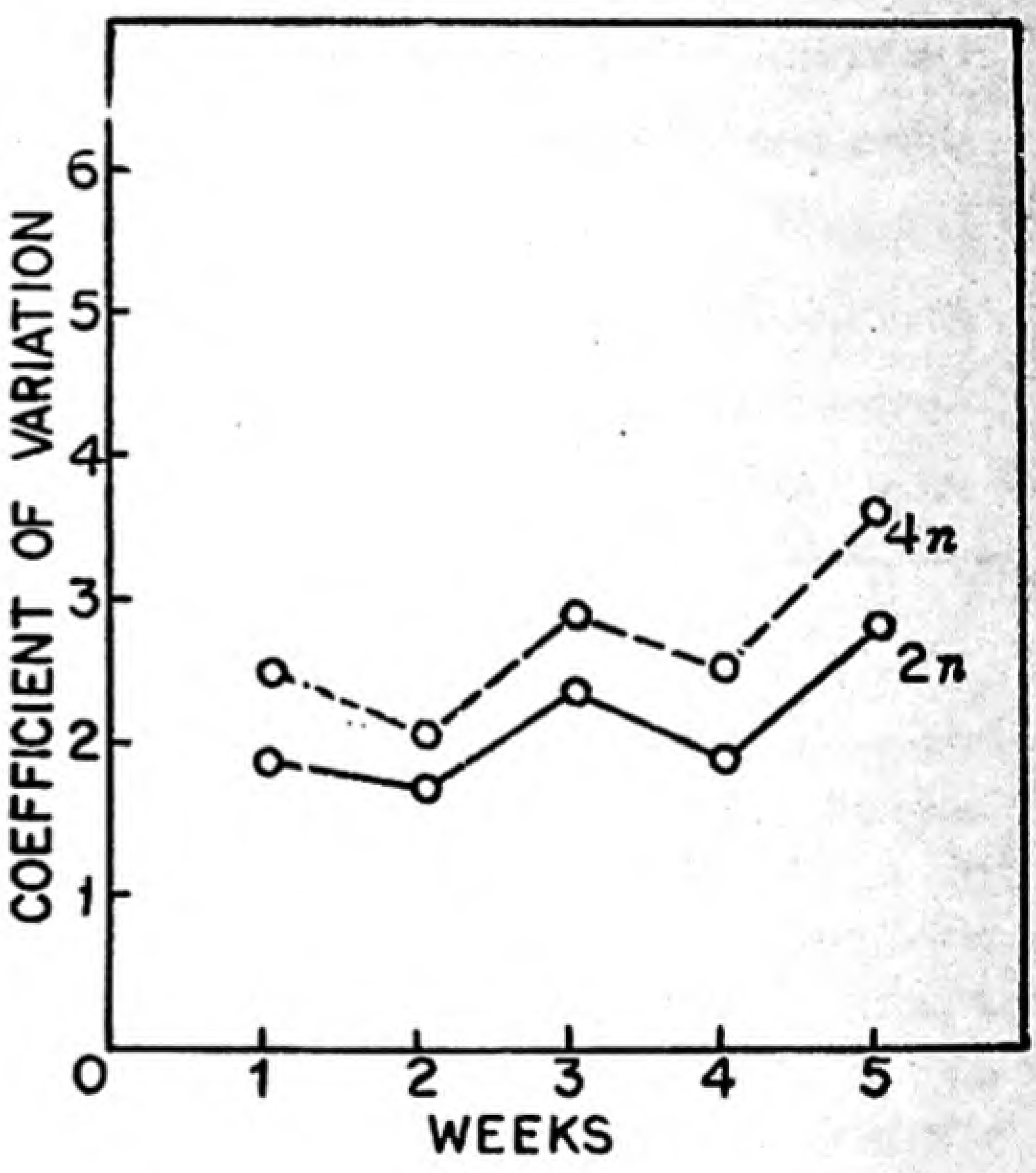
*Significant at 0.05 level.

Cross and Johnson (1941) while working with *Vinca rosea* indicated that the increased width of the tetraploid species was due to the increased lateral diameter of cells in the apical meristem. At the same time the vertical diameter was not increased much in proportion.

Morphological characters.—Considerable increase in the thickness of the leaves was noticed in the first few leaves in the case of *R. serpentina* and it was interesting to note that it had persisted in other leaves also. This indicated successful induction of tetraploidy in the treated seeds and seedlings.



RATE OF GROWTH OF 2n AND 4n



Flowers were found to be less in number per inflorescence in the tetraploid as compared with those of the diploid *R. serpentina*. Schidt and Ekerberg (1951) attributed the lesser flower initials to a lower cell number in rye. Sen and Chedda (1959) while working with black gram observed an inflorescence axis to be shorter in length and with lesser number of flowers thereon.

Floral parts in the tetraploid were found to be shorter in length but thicker in diameter when compared with those of the diploid *R. serpentina*. Similar types of observations have been recorded by several workers. (Gorenflot, 1960; Sen and Vidyabhushan, 1960; Sanudo, 1962).

Delayed flowering was observed in the tetraploid of *R. serpentina*. It was also recorded by Sen and Vidyabhushan (1960) in *Dolichos biflora*. It was attributed by them like other physiological phenomena, to the slower rate of metabolic activity and their continued flowering had probably been due to less exhaustion of the plants in fruit setting, which was usually reduced.

Root and alkaloid content.—The maximum amount of fresh root weight per diploid plant of *R. serpentina* was 56.7 g and the minimum was 50.9 g. The roots were slightly thicker and more branched than the roots of the diploid. In none of the cases roots were found to be fleshy. Bhaduri and Biswas (1965) claimed to have obtained 414 g. of fresh root weight of tetraploid of *R. serpentina* and the minimum weight as 198.1 g. They found that the roots were also more bulky and fleshy.

Out of 80 species of *Rauvolfia* a very few species contain reserpine. Another species which yielded larger quantities of the alkaloid reserpine (0.09%) was found to be the African species *R. vomitoria* (McAleer et al., 1956). But according to Hamied and Bakshi (1956), Indian *R. serpentina* roots contain 0.11% of reserpine. The present authors also found the same percentage (0.11%) of reserpine while assaying the roots. The total alkaloid content with reserpine was 1.81% (total alkaloids B. P. C. =1.58%) in the diploid while it was 2.01% in the tetraploid. 11% increase in the total alkaloid content in the tetraploid is quite appreciable. The percentage of reserpine content in the tetraploid was, however, more or less same (0.11%) as in the diploid.

As *R. serpentina* plants propagate by stem cuttings. it will be easier to increase the number of tetraploids on a large scale from a few successfully induced ones. If this method of cultivation of tetraploids is followed successively for at least three generations and if the percentages of total alkaloid content and reserpine are found almost constant in them as authors had observed on an experimental basis, the extensive cultivation of such a particular tetraploid clone in collaboration with the forest department promises to solve the present problem of the pharamaceutical industry which requires roots of better quality with uniform performance.

Karyotype.—The diploid plant of *R. serpentina* has two SAT-chromosomes (Dnyansagar and Torne, 1967) while the tetraploid (induced and natural) has four SAT-chromosomes: Bhattacharjee and Bhaduri (1959) have mentioned the presence of six SAT-chromosomes in the diploid of *R. serpentina* but have not stated anything about the SAT-chromosomes in the autotetraploid of this plant. Blackburn (1933) has observed that the diploid race of *Silene ciliata* has two SAT-chromosomes while the tetraploid has four SAT-chromsomes. Dnyansagar and Torne (1966) have found one plant of *R. tetraphylla* (syn. *R. canescens*) where the chromosome number determined is $2n=44$, besides other *R. tetraphylla* where the number is $2n=66$ with one pair of SAT-chromosomes.

In the genus *Rauvolfia*. Raghavan (1957) has detected polyploid series. *In R. serpentina, R. densiflora* and *R. canescens* he has observed $2n=22$, $2n=44$ and $2n=66$ chromsomes, respectively. He has considered *R. serpentina* as a diploid and *R. densiflora* and *R. canescens* representing tetraploid and hexaploid levels, respectively.

The authors had assayed amino acids in the leaves of the diploid, induced and natural tetraploid plants of *R. serpentina* and it was observed that all the amino acids present in the leaves of the diploid were also present in leaves of the induced and natural tetraploids. Taking into consideration this fact as well as the similarity of karyotypes of the diploid induced and natural tetraploids, it can be concluded that the natural tetraploid is an autotetraploid. So *R. serpentina* possesses both the diploid and tetraploid plants.

Meiosis.—The presence of multivalents in the tetraploid indicates a high degree of homology of more than two chromosomes. Accompanying the variability of chromosome associations is a variability in the number of chiasmata. In all plants studied, the chiasma frequency in the tetraploid (induced and natural) has never equalled twice that of the diploid from which it was derived. This drop in number arises because there are usually only 4 chiasmata for each zig-zag, ring and open quadrivalent, and the equivalent number as the bivalent will be five or six.

There were fewer quadrivalents than bivalents in the natural tetraploid while in the induced tetraploid quadrivalents exceeded bivalents in number. The value of correlation coefficient of quadrivalents and bivalents of the natural tetraploid is more as compared to that of the induced tetraploid thus suggesting that colchicine has affected the number of quadrivalents by increasing their number in the induced tetraploid. Giles and Randolph (1951) observed a similar type of result in maize.

The cytological data obtained during the present study indicate that no correlation exists between the univalent frequency and seed fertility or between the multivalent frequency and seed fertility. Plants with a high multivalent frequency were found to be as much seed fertile as plants with a low multivalent frequency. This may probably be due to the fact that there is a high proportion of symmetrically arranged quadrivalents. It, therefore, seems probable that the multivalent types rather than the frequency determine the fertility in the present material. Sparrow et al. (1942) from a study of inter- and intravarietal tetraploids of *Antirrhinum majus* found that the varying fertility in different crosses was not correlated with cytologial behaviour. Armstrong and Robertson (1956) in the course of their studies on induced tetraploids of alsike clover observed that cytological behaviour was not closely associated with varying fertility.

The chiasma frequency and coefficient of terminalization in the natural tetraploid were 1.54 and 0.5, respectively while for the induced tetraploid the figures were 1.67 and 0.43 respectively. The natural tetraploid has less chiasma frequnency and hence fewer quadrivalents as compared with the induced fertraploid (Morrison and Rajhathy, 1960). According to Kostoff (1940) plants with smaller chromosomes have fewer quadrivalents.

Aberrant spindles are formed in tetraploids of *R. serpentina*. Walters (1958), Beadle and McClintock (1928) have said that aberrant spindles occur at meiosis in plants in which large numbers of univalents are present. But Heitz (1926), Swanson and Nelson (1942) have reported the occurrence of aberrant spindles in certain plants containing regularly paired chromosomes. In tetraploids of *R. serpentina* univalents are rare and their number never exceeds two. Walters (1958) has suggested the origin of multipolar spindles from splitting or extra division of spindle organizers in the first or second prophase.

Meiosis was disturbed in an appreciable number of pollen mother cells of tetraploids of *R. serpentina* and supernumarary cells were also observed at the tetrad stage. Hence an appreciable proportion of pollen grains must have aberrant chromosome numbers. Many of these aberrant pollen grains must have been aborted since only exactly tetraploid progeny was obtained or if they functioned there must be zygotic elimination of the embryos. Of course, some embryo sacs and eggs should also have aberrant chromosome numbers too, and be non-functional. Since there are regularly 2 ovules in each ovary and usually one develops into a seed, such elimination may not seriously affect the seed production. It would seem that there is no real scarcity of pollen grains and ovules since no correlation has been found between the frequencies of the meiotic irregularities which produced the aberrant chromosome numbers in the gametes and the amount of seeds produced by the tetraploid plant of *R. serpentina*. Meiosis in megaspore mother cells of tetraploids of *R. serpentina* should be about as irregular as in pollen mother cells. Presumbaly, mostly such aberrant megaspores lead to the ovule abortion before or during the embryo-sac development, or result in the non-functional embryo-sacs. This type of ovule elimination is likely to occur at the same time in both ovules of one ovary frequently enough to reduce seed production to some extent. This should hold good as regularly one ovule is lost per ovary. If genetic factors also operate to cause the ovule abortion, as they apparently do to cause the pollen abortion, the low fertility of tetraploid *R. serpentina* plants is explainable. Furthermore, if some of the genetic factors which cause pollen abortion also cause the ovule abortion, the correlation between the extent of the pollen abortion and the amount of seed set is understandable. Chromosomal and genetic factors causing reduced fertility will be much more effective in the female side of reproduction than in the male where there is an abundance of normal pollen and strong competition among the numerous pollen grains available to effect fertilization.

References

Armstrong, J.M., and R. W. Robertson. 1956. Studies of C-induced tetraploids of *Trifolium hybridum*. L. I. Cross and self-incompatibility and cytological observations. *Can. J. agri. Sci.* **36:** 255-266.

Beadle, G. W., and B. McClintock. 1928. A genic disturbance of meiosis in *Zea mays*. *Science, N. Y.* **68:** 433.

Bhaduri, P. N., and P. K. Biswas. 1965. Increasing root yield of *Rauvolfia serpentina* by colchiploidy. *Sci. Cult.* **31:** 197-200.

Bhattacharjee, S. K., and P. N. Bhaduri. 1959. Floral biology and cytogenetics of diploid and autopolyploid *Rauvolfia serpentina* and *R. canescens*, *Bull. bot. Soc. Bengal* **13:** 86-92.

Blackburn, B. 1933. On the relation between geographic races and polyploidy in *Silene citrate* Pourr. *Genetics* **14:** 449-466.

Cross, G. L., and T. J. Johnson. 1941. Structural features of the shoot apices of diploid and colchicine induced tetraploid strains of *Vinca rosea*. L. *Bull. Torrey bot. Club*. **68:** 618-635.

Dnyansagar, V. R., and S. G. Torne. 1966. Karyotype analysis of *Rauvolfia serpentina* Benth. and *R. tetraphylla* L. *Proc. 54th Indian Sci. Congr. Part III* (Abst.) 395.

——, and ——. 1967. Mitosis and karyotype analysis of *Rauvolfia serpentina*. *J. biol. Sci.* **10:** 7-13.

——, and ——. 1969. Meiotic analysis in *Rauvolfia serpentina* (L.) Benth. ex Kurz. *Proc. natn. Inst. Sci. India* **35B:** 368-376.

Eigesti, C. J., and L. Schnell. 1943. A comparison of colchicine treatment with glycamine base and a water base. *Genetics* **28:** 73.

Erdtman, G. 1952. *Pollen Morphology and Plant Taxonomy*. Almqvist and Wiksell, Stockholm.

Furusato, K. 1940. Polyploid plants produced by colchicine. *Bot. Zool.* **8:** 1303-1311.

Giles, A., and L. F. Randolph. 1951. Reduction of quadrivalent frequency in autotetraploid maize. *Am. J. Bot.* **38:** 12-17.

Gorenflot, R. 1960. Polyploid in *Plantago coronopus* L. *Rev. cytol. biol. vég.* **22:** 77-108.

Hamied, K. A., and V. M. Bakshi. 1956. *Rauvolfia serpentina* alkaloids. New method of assey. *Indian J. Pharm.* **18:** 190-192.

Hawkies, J. 1942. Some effects of the drug colchicine in cell division. *J. Genet.* **44:** 11-12.

Heitz, E. 1926. Beiträge Zur cytologie von *Melandrium*. *Planta* **1:** 241 : 259.

Janaki Ammal, E. K. 1962. Tetraploidy in *Rauvolfia serpentina* Benth. *Curr. Sci.* **31:** 520-521.

Koltzeff, N.K. 1939. On the methods of artificially inducing polyploids by treatment with colchicine. *C. r. (Doklady) Acad. Sci. URSS* **23:** 482-485.

Kostoff, D. 1940. Fertility and chromosome length. *J. Hered.* **31:** 33-34.

Levan, A. 1938. The effect of colchicine on root mitoses in *Allium*. *Hereditas* **24:** 470-486.

McAleer, W. J., R. G. Weston, and E. E. Howe. 1956. Plant sources of reserpine. *Chem. and Indust.* 1387.

MORRISON, J. W., AND T. RAJHATHY, 1960. Frequency of quadrivalents in autotetraploid plants. *Nature, Lond.* **187**: 528-530.

NAIR, P. K. K. 1965. Pollen grains of Indian Plants-I. *Bull. natn. bot. Gdns.* No. 53.

RAGHAVAN, R. S. 1957. Chromosome numbers in Indian medicinal plants. *Proc. Indian Acad. Sci.* **45B**: 294-298.

SANUDO, A. P. 1962. Cytogenetic studies in the genus *Solanum*, series *Cardiophylla* and *Pinnatisecta*. II. Artificial polyploids: Autotetraploids and amphidiploids. *An Inst. Nac. Invest. Agron.* **11**: 157.

SCHIDT, R., AND E. EKERBERG. 1951. Studies of tetraploid and diploid rye at the Ultuna Branch station in 1949. *Sver. Utsädesför. Tidskr.* **61**: 254.

SEN, N. K., AND H. R. CHEDDA. 1958. Colchicine induced tetraploids of five varieties of black grass. *Indian J. Genet. Pl. Bread.* **18**: 238-248.

——, AND K. V. VIDYABHUSHAN. 1960. Studies on the induced polyploids of horse grass. *Indian J. Genet. Pl. Breed.* **20**: 212-222.

SPARROW, A. H., M. L. RUTTLE, AND B. R. NELEL. 1942. Comparative cytology of sterile intra- and fertile inter-varietal tetraploids of *Antirrhinum majus* L. *Am. J. Bot.* **29**: 711-715.

SWANSON, C. P., AND R. NELSON. 1942. Spindle abnormalities in *Mentha*. *Bot. Gaz.* **104**: 243-280.

WALTERS, M. S. 1958. Aberrant chromosome movement and spindle formation in meiosis of *Bromus hybrids*. An interpretation of spindle organization. *Am. J. Bot.* **45**: 271-289.

Adv. Pl. Morph. 1972: 193-202

SPECIATION IN RELATION TO THE BREEDING SYSTEM IN THE CUCURBITACEAE[1]

BY R. P. ROY, SUNIL SARAN AND BITHI DUTT

Department of Botany, Patna University, Patna-5, India.

ABSTRACT

Based on studies in six genera, speciation in the Cucurbitaceae has been discussed in relation to the breeding system. Members of this family incorporated a variety of sex forms showing a mode of reproduction ranging from partial autogamy to obligate allogamy. A number of evolutionary trends are indicated from this study among the cucurbits. These are :—(i) Polyploidy plays a little or no role in speciation. (ii) Obligately outcrossed plants show a lower chiasma frequency as compared to the partially autogamous plants. (iii) Bees as pollen vectors prevents interspecific pollinations among the sympatric species and impose a sort of premating isolation. (iv) Separation of sexes in the dioecious forms has been both genic as well as chromosomal, and the evolution of dioecism appears to have followed the generally accepted line developing from hermaphroditism through monoecious types. *Luffa* may be an exception.

INTRODUCTION

In order to interpret the evolutionary dynamics of a plant group, an understanding of its breeding system as well as the morphological features associated with its mode of reproduction is of utmost importance. The overall reproductive potentiality of any group controls the spread of genetic variability, and reflects its evolutionary trends. Variability in its turn controls the rapidity of adaptive change. In other words, the capacity for genetic response to selection depends upon the provision of variability (Simmonds, 1962). The pollinating system of a plant group is equally important, since it delimits the type of mating system which occurs naturally. Once the new types arrive either through recombination or mutation, their maintenance will largely depend upon the mode of propagation of the species. An outbreeding system has a certain selective advantage since it promotes gene recombination that plays as great a role as mutation in providing variability on which the natural selection will work. On the other hand inbred plants as well as obligately apomicts produce greater and more secure seeds as compared to the outbred plants but at the cost of genetic

---

[1] This research has been financed in part by a grant made by the United States Department of Agriculture under PL 480.

variability due to restriction or elimination of segregation and recombination. There are some excellent contributions by workers like Baker (1953, 1955, 1959, 1961), Stebbins (1950, 1957), Grant (1949, 1956, 1958), Fryxell (1957), and Simmonds (1962) dealing with reproductive methods and evolution in higher plants. Broadly speaking, the higher plants reproduce either sexually (amphimixis) or asexually (apomixis). The sexually reproduced plants may be habitually outbreeding (allogamous), inbreeding (autogamous), or show a combination of both. All cross-pollinated plants come under the category of the outbred type with a number of contrivances supporting this type of breeding system. The dichogamous plants with protogyny and protandry are always cross-pollinated. The dioecious plants, where outcrossing is obligatory, show the most drastic type of allogamous system. Gynodioecism is another mechanism favouring outcrossing since the hermaphrodite flowers may be selfed, but the female flowers must be outcrossed. Therefore, on the same individual, presence of different mating systems will contribute unequal number of genes to the next generation (Lewis and Crowe, 1956). As opposed to the allogamous plants, the habitually inbred plants set seeds as a result of self-pollination, although, according to Baker (1959), there is probably no inbreeder which does not ever occasionally outbreed. For self-fertilization, cleistogamy forms the best device. In a population, inbreeding leads to increased homozygosity resulting in a lack of genetic variability which may be advantageous in a stable environment, but is highly disadvantageous in a changing environment since the population will lack the capacity for change to meet new conditions. As has been pointed out by Mather (1956), homozygotes are "less able to withstand the vagaries of environmental upset and developmental accident". According to Simmonds (1962), however, for evolution and detection of newly evolved variability the most suitable system is provided by the casually out-pollinated inbreeder. The asexually reproducing plants propagate either vegetatively or through agamospermy. Individuals produced vegetatively by bulbils, tubers, rhizomes, etc., are important from the genetic and evolutionary points of view because they have the same genotype and consequently belong to the same biotype. Agamospermy represents a specialized process for the production of embryo and seeds non-sexually. The obligate type of a agamospermous plants produce individuals which constitute a clone of the same biotype representing a system genetically similar to the vegetatively reproduced plants. According to Hutchinson (1965), loss of sexual reproduction in a plant brings evolution to a halt, and mutation brings about diversity in the clones. As opposed to this type of a closed evolutionary system, the facultatively apomictic plants which incorporate a dual system for reproduction, sexual and asexual, represent a highly labile evolutionary system. As pointed out by Clausen (1961) and Harlan (1963), facultative apomixis provides an ideal opportunity for massive introgression leading to enormous variability. It is evident from the above review that the type of breeding system will have an important bearing on the evolutionary trends of a plant group.

Breeding system in the Cucurbitaceae.— The cucurbits dispay a variety of sex forms. Majority of plants are monoecious, a few are dioecious, and relatively fewer bear hermaphroditic flowers. Apart from these stable sexual forms, there are members in the Cucurbitaceae that exhibit a number of intermediate forms, such as, andromonoecious, gynomonoecious, trimonoecious and gynodioecious types. All cucurbits are entomophillous. Flowers which open in the evening are pollinated by nocturnal bees while those that open in the morning are visited by honey bees and ants. With regard to the breeding system, the family comprises of species that are partially self-, partially crossfertilized, or are principally or obligately cross-fertilized. Asexual reproduction, although not very common among the cucurbits, is known in a few genera. Species like *Trichosanthes cordata, Coccinia indica, Momordica dioica, Gomphogyne cissiformis,* and *Cucurbita radicans* reproduce vegetatively by perennating tubers. The present attempt to examine speciation in the Cucurbitaceae in relation to the breeding system will be based on genera that have been under investigation in this laboratory for several years now. These are *Luffa, Momordica, Trichosanthes, Cucumis,* and *Coccinia*. The genus *Cucurbita* has also been included in this study as a representative from the New World.

Luffa.—The genus *Luffa* has four stable species, namely, *L. cylindrica, L. acutangula, L. graveolens* and *L. echinata*. All are diploids with $2n=26$. Of the four species, the first two named are cultivated and the last two are wild. There are several sex forms in this genus; *L. cylindrica* and *L. graveolens* are strictly monoecious, *L. echinata* is dioecious, and *L. acutangula* which is monoecious has a cultivar 'Satputia' which bears perfect flowers. Other sex forms in *Luffa* are known from intervarietal and interspecific crosses. Choudhury and Thakur (1965) obtained andromonoecious and gynoecious plants in the progeny of intervarietal crosses in *L. acutangula*. They also obtained andromonoecious and trimonoecious plants from the inter specific cross between *L. cylindrica*, and *L. acutangula*. In an interspecific cross between *L. echinata* and *L. graveolens*, Dutt and Roy (1971) reported the occurrence of a few androecious plants.

The most common method of reproduction in *Luffa* is represented by the monoecious species where plants are partially self- and partially cross-fertilized. From this type of a breeding system two lines of variations are observed. One line is represented by the dioecious species *L. echinata* where outcrossing is obligate, and the other line by the cultivar 'Satputia' of *L. acutangula*, with perfect flowers, where self-fertilization is relatively favoured. Other variants like the andromonoecious and the, gynomonoecious forms, where stable, show various degrees of self and cross fertilization. In the monoecious forms cross-pollination is favoured, but outcrossing is not guaranteed.

The pollen vectors in the cross-fertilized members of *Luffa* are bees and ants. A fairly rigid within-the-species pollination is enforced by the pollinating agents with the result that no natural hybrids are observed where different species are growing in the same locality. When *L. acutangula* and *L. cylindrica* are crossed artificially, about 30-35% of pollen fertility is observed in the F_1 hybrids, but in nature these species do not cross even when they grow side by side (Roy and Trivedi, to be published). In nature, therefore, the four species of *Luffa* appear to be sexually well isolated. A study of recombination in these species, as revealed by the chiasma frequency, shows that *L. acutangula*, which is partly autogamous, has higher chiasma frequency

(25.40 per PMC at metaphase I) as compared to *L. echinata* (24.50) which is entirely outcrossed.

The differentiation of sex in the dioecious species *L. echinata* is genic since all chromosome pairs are homomorphic.

On the basis of detailed cytogenetical studies in *Luffa*, Dutt and Roy (1971) have concluded that *L. graveolens* forms the prime species which evolved along two lines. Along one line it gave rise to the two cultivated species, *L. cylindrica* and *L. acutangula* while along the other line *L. echinata* evolved. Emergence of *L. echinata* has been associated with the evolution of dioecism. Karyotypic differences were also involved in the evolution of species in *Luffa*.

Momordica.—Three species of *Momordica* have been investigated in detail cytogenetically in this laboratory. Of these three species, *M. charantia*, and *M. balsamina*, both with $2n=22$, are monoecious, and *M. dioica* with $2n=28$ is dioecious. *M. charantia* and *M. balsamina* are partly auto-, partly allogamous while *M. dioica* is obligately allogamous. Karyotype analysis revealed that *M. dioica* possessed a greater asymmetry of chromosomes as compared to the other two species showing that it was more advanced. Important features noted in *M. dioica* which is also perennial were its: (a) long generation, (b) high chromosome number, (c) low chiasma frequency, and (d) obligate outcrossing. On the other hand, the other two strictly monoecious and annual species, *M. charantia* and *M. balsamina*, showed: (a) short generation, (b) low chromosome number, (c) high chiasma frequency, and (d) partial autogamy.

Attempts were made to cross *M. charantia* and *M. balsamina* with *M. dioica* in both directions, but none of the over 400 pollination attempts did succeed. In these crossing attempts even the germination of the pollen grains was markedly scanty. It was concluded from these studies that incompatibility barrier between 22- and 28-chromosome species in *Momordica* was complete and stable (Trivedi and Roy, 1972).

In the evolution of *M. dioica*, where the differentiation of sex is genic, three trends were well marked: (i) asymmetry of chromosomes, (ii) an ascending dysploidy, and (iii) dioecism.

Trichosanthes.—Four species have been studied in detail in the genus *Trichosanthes*. Out of these, *T. cucumerina*, *T. anguina*, and *T. lobata* are monoecious, and *T. dioica* is dioecious. All are diploids with $2n=22$. The monoecious, species are partly auto-, partly allogamous whereas, the dioecious species is obligately allogamous. The monoecious species show a higher chiasma frequency in comparison to the dioecious species. In *T. cucumerina* the chisama frequency per PMC at metaphase I is 21.00, whereas, in *T. dioica* it is slightly less being 20.80 (Roy and Trivedi, to be published).

Hybridization studies show that *T. cucumerina*, *T. anguina*, and *T. lobata* are cross compatible, and their taxonomic status needs to be revised. On the other hand *T. dioica* is sexually isolated from the other species.

In *T. dioica* the differentiation of sex is genic rather than chromosomal. Patel (1952) had reported a pair of heteromorphic chromosomes in *T. dioica* which was associated with dioecism. Studies in this laboratory failed to reveal any chromosomal differentiation of sex in this species. From the studies of Singh (1953) indications are available regarding evolution of dioecism in *Trichosanthes*. He obtained aberrant plants of *T. anguina* and *T. dioica* that developed some hermaphroditic flowers but they were all sterile. These aberrant types were referred to as cases of reversal to the ancestral form. It is reasonable to conclude that *T. dioica* evolved from a hermaphrodite ancestor through monoecism which is the most common habit in this genus, and later became sexually isolated from other species.

Coccinia.—Only one species of this genus, *C. indica,* occurs in India. This species which has been well worked out cytologically (Kumar and Deodikar, 1940; Bhaduri and Bose, 1947; Roy and Roy, 1970) is strictly dioecious showing an obligate allogamy. So far, it is also the only confirmed member among the dioecious cucurbits to possess a chromosomal differentiation of sex of the X/Y type. On the basis of artificially raised polyploids and crossing experiments between them, Roy and Roy (1970) furnished further details regarding dioecism in this species. They have found that in *C. indica* polyploidy is not a barrier to dioecism, although natural polyploids are unknown in this species. They also found that a plant with the genotype XXXY+4A was male showing thereby that the Y chromosome in this species was strongly male determining.

It is interesting to note that Kumar and Vishveshwaraiah (1952) discovered a gynodioecious plant in *C. indica* with a chromosome constitution of XX+2A. Although this plant had developed anthers, but due to lack of pollen formation maintained obligate outcrossing typical of the species. This aberrant type may also be looked upon as a case of reversal to the ancestral hermaphroditic habit and represents an important step in the evolution of dioecism.

The X/Y type of sex mechanism found in *C. indica* represents a step ahead in the evolution of dioecism in comparison to the genic differentiation of sex found in other dioecious cucurbits. Due to restriction of crossing-over in the heteromorphic sex chromosome dioecism is maintained. According to Westergaard (1958), however, in such a system the Y chromosome, being unpaired, is highly susceptible to mutations and tends to become inert. In such a situation selection will favour a genotype where the Y chromosome is rendered superfluous, and sex is determined by a balance between Y chromosome and the autosomes (X/Y type). In this regard the X/Y system in *C. indica* represents an intermediate type in evolution.

Cucumis.—Some interesting studies have been made in *Cucumis sativus* relative to the development of morphological forms connected with sex expression, and these findings represent important steps in the evolution of dioecism which characteristically alters the breeding system of a species. *C. sativus* is predominantly monoecious, but includes other sex variants. The sex forms found in this species, apart from the monoecious condition, are of gynoecious andromonoecious, and hermaphrodite types. The monoecious forms further consist of strictly male, mixed, and strictly female phases in a progressive order. According to Tkachenko (1935) in cucumbers a rare gene exists which is often associated with complete femaleness causing developmentally persistent gynoecism. Shifriss and Galun (1956) provided evidence to show that this gene did not control gynoecism, but simply accelerated the rate of sex conversion, particularly femaleness. This gene was designated as *Acr* (Shirfriss, 1961). With the establishment of the accelerating gene, two gynodioecious races were synthesized by incorporating *Acr* into diverse genetic backgrounds (Shifriss *et al.*, 1964). These synthetic forms as well as the presence in nature of the *Acr* gene provide important clues to the origin and evolution of dioecism.

Depending upon the type of sex form, the breeding system in *C. sativus* varies from predominantly autogamous to essentially allogamous type.

Cucurbita.—The New World genus *Cucurbita* comprises of 5 cultivated and 19 wild species (Whitaker and Bemis, 1964). All species are diploids with $2n = 40$. They are all monoecious and partially self-, partially cross-fertilized with bees acting as pollen vectors. On the basis of hybridization studies, Whitaker (1956) concluded that the cultivated species in nature were cross-incompatible among themselves and presented a strong barrier to interspecific crosses. On the other hand, all the cultivated species showed cross-compatibility with the wild species, *C. lundelliana*. Hurd and Linsley (1964) presented breeding data to show that bees as pollen vectors in the xerophytic species of *Cucurbita* exhibited certain specific preferences. As a result of this, cross-pollination by bees was restricted within the species which helped maintain a premating isolation preventing interspecific hybridization, thus dividing the xerophytic species into two groups.

DISCUSSION AND CONCLUSIONS

If an attempt is made to examine speciation in the Cucurbitaceae in response to the system of breeding, certain evolutionary trends become quite evident. Although the present conclusions are based on studies extending over selected species in six genera, the findings are significant enough to shed a valuable light on speciation in the family as a whole.

Asexual reproduction is rather limited in the cucurbits, and only of the vegetative type with the plants propagating principally by perennating tubers. Moreover, vegetative reproduction in this family is not of obligate nature since plants that propagate asexually are basically amphimictic. Therefore, asexuality does not appear to affect the course of evolution in the Cucurbitaceae in any significant way other than maintaining in clones the variability conferred by sexuality. Evolutionary trends in the family therefore, have to be sought in the manner through which sexuality operates and is enforced various breeding and pollinating systems.

By and large, a majority of cucurbits display partial auto-, partial allogamy with the degree or the extremes of one or the other type determined by the sex forms which in turn may be quite varied showing different categories of hermaphroditism, monoecism, and dioecism. The extreme type is provided by the dioecious plants where outcrossing is obligatory.

Polyploidy is rare among the cucurbits and appears to have played little or no role in speciation in this family. The cucurbits being principally cross-fertilized plants, this is expected. As has been pointed out by Grant (1956), who has cited examples from *Layia, Madia, Gilia* etc., the obligately cross-fertilized plants are strictly diploid as opposed to the self-fertilizing species which may contain several allopolyploids. This has been explained on the assumption that where time available for reproduction by a natural hybrid is critical as in the annuals, biennials, and short-lived perennials, under conditions of self-fertilization natural hybridization will tend to run towards polyploidy, whereas, habitual allogamy will lead towards introgression. Grant (1958) has further mentioned that in a cross-fertilizing groups, the hybrids tend to backcross to the parents rather than intercross with sister hybrids. This happens due to the abundance of the parental individuals and higher fertility of the backcross progeny. As a result of this the diploid level of the species is retained. For polyploidy to develop in a cross-fertilized group it will be necessary that the two gametes coming in contact for fertilization be unreduced which will be very rare (Stebbins, 1957).

Both in our own studies in *Luffa*, and in the investigations of Whitaker (1956) in *Cucurbita*, it was observed that the cultivated species growing in the same locality do not show interbreeding and maintain a stable barrier against interspecific hybridization as evidenced by a lack of hybrids in such sympatric populations. Occasional introgression may take place but on account of repeated backcrossing, the progeny resembles the recurrent parent so strongly that it is difficult sometimes to detect the effect of natural hybridization. As has been pointed out by Anderson (1953), in such populations the effect of natural hybridization remains cryptic. It is reasonable to assume that stable sexual isolation develops between different sympatric species because they are close enough to produce deleterious hybrids. On the other hand, sexual isolation between allopatric populations is weak as demonstrated by Whitaker (1956) in *Cucurbita* where cultivated species are cross compatible with the wild species *C. lundelliana*. Similarly, Grant (1965) has shown in case of *Gilia* that sympatric species are most difficult to cross, but in allopatric species there is no barrier to hybridization although all crosses are sterile.

The pollen vectors in the cross-fertilized cucurbits are primarily bees and ants. The bees show a remarkable intraspecific preference as pollinating agents, and pose an ethological barrier to prevent interspecific hybridization (Grant, 1949). In *Luffa* and *Cucurbita* the bees as pollinating agents, successfully prevent interbreeding between cultivated species growing in the same locality. In *Luffa*, the cultivated species *L. cylindrica* and *L. acutangula* show strict sterility barriers to interspecific crossing in nature, but when hand pollinated, the F_1 hybrids show 30-35% pollen fertility. According to Anderson (1948), in higher plants, species growing in nature in the same range without evident hybridization, may produce fertile progeny in the breeding plot. This is evidently due to the preferential behaviour of the pollinating agents. The productive isolation among sympatric populations of different species may be primarily genetic which is further reinforced by within-the-species pollinating preference of the bees. Ethological isolation is considered to be more important in augmenting allopatric speciation rather than initiating sympatric divergence (Grant, 1949). Bee pollination further imposes a kind of premating isolation (Mayr, 1963). The genotypes that incorporate this kind of premating isolating mechanism have the advantage of preventing wastage of gametes in the production of deleterious hybrid offspring. For this mode of speciation to be effective it is necessary that the species producing deleterious hybrids are exposed to each other in the same locality. Only under such situations the more sexually isolated genotypes are specifically selected. The cucurbits appear to have adopted this type of mating system to the best advantage in speciation.

Commenting on the problem of retaining adaptive stability among the outbreeders, Baker (1953) mentions that, "adaptive success goes to those outbreeders, therefore, with reduced recombination, thus retaining heterozygosity while restricting variation." The rate of recombination is reflected in the chiasma frequency, therefore, the outbreeders should show a lower chiasma frequency as compared to the inbreeders. A comparison of chiasma frequencies in *Luffa, Momordica,* and *Trichosanthes* between dioecious and monoecious species reveals that the dioecious species where outbreeding is obligate invariably show a lower chiasma frequency as compared to the monoecious species where partial autogamy may be present.

Dioecism represents an important line of evolution in the cucurbits. Both genetical as well as cytological differentiations determining sex in the dioecious members of the Cucurbitaceae are known. Three of the four species included in this study, namely, *Luffa echinata, Momordica dioica,* and *Trichosanthes dioica* show differentiation of sex at the genic level, whereas, *Coccinia indica* has been conclusively demonstrated to possess an X/Y type sex mechanism and probably the only cucurbit to show heteromorphic sex chromosome. The system for sex determination gets established in plants leading to dioecism through mutations, and restriction of crossing-over facilitated further by heteromorphic chromosomes (Westergaard, 1958; George, 1970). Both in plants and animals it is generally recognised that the hermaphrodite sexual form represents the primitive condition from which the evolution has been towards the separation of sexes. The hermaphroditic condition thus represents the ancestral type from which the monoecious forms have developed. The monoecious forms in turn give rise to the dioecious forms. In the Cucurbitaceae this conclusion is substantiated by the occasional reversal of both dioecious and monoecious plants to the hermaphroditic or gynodioecious conditions as reported in the aberrant plants of *Trichosanthes dioica* and *T. anguina* (Singh, 1953), *Coccinia indica* (Kumar and Vishveshwaraiah, 1952), and *C. sativus* (George, 1970). Conversely, in our own studies it was found that *L. acutangula* and *Momordica charantia,* both monoecious, gave rise to purely gynoecious plants indicating the beginning of dioecism. In *Luffa*, according to Singh *et al.* (1948), Richharia (1948), and Thakur and Choudhury (1966), the various sex forms are controlled by two genes A and G. The gene A converts most perfect flowers into pistillate ones, whereas, G converts most perfect flowers into staminate. Monoecious forms develop when both the genes are present (AG). Gynoecious forms possess a genotype Ag, and the androecious forms are aG. The double recessive genotype (ag) gives rise to the perfect flowers. In this type of a genotypic background, the origin of dioecism can be easily visualised to have resulted due to mutation in either of A or G. The *Acr* gene in *Cucumis sativus* suggests a similar line in the origin and evolution of dioecism. The evolution of sex forms in *Luffa*, further presents an intriguing situation especially with regard to the cultivar 'Satputia' of *L. acutangula* which is a true breeding hermaphrodite. The genotype of this cultivar has been found to possess two independent double recessive loci (ag). It is difficult to say that this double recessive type has developed as a reversal mutant from the monoecious condition of *L. acutangula* and represents a primitive condition. If not, we have to accept that the hermaphroditic condition in 'Satputia' is a stable and derived form from the monoecious condition. Until further genetic data are furnished to the contrary, it may be mentioned that *Luffa* represent a situation where both dioecious and hermaphroditic forms appear to have been derived from the monoecious condition.

In conclusion it may be stated that the breeding system in the Cucurbitaceae, which is basically either facultative or obligate outcrossing, exerts an important influence in speciation. The sympatric species get strongly isolated reproductively due to cross incompatibility which is further strengthened by system of premating isolation imposed by bee pollination which prevents interspecific pollinations. Control of recombination leads to adaptive stability in a given environment. Evolution of dioecism provides another line for speciation. In this family polyploidy is rare and appears to be of little or no evolutionary consequence.

REFERENCES

Anderson, E. 1948. Hybridization of the habitat *Evolution* **2:** 1-9.

——. 1953. Introgressive Hybridization. *Biol. Rev.* **28:** 280-307.

Baker, H. G. 1953. Race formation and reproductive method in flowering plants. *Symp. Soc. Exp. Bio.* **7:** 114-115.

——. 1955. Self compatibility and establishment after "long distance" dispersal. *Evolution* **9:** 347-348.

——. 1959. Reproductive methods as factors in speciation in flowering plants. *Cold Spr. Harbor. Symp. Quant. Biol.* **24:** 177-191.

——. 1961. Rapid speciation in relation to changes in the breeding system of plants. In *Recent Advances in Botany*. Toronto Univ. Press. 881-885.

Bhaduri, P. N., and P. C. Bose. 1947. Cytogenetical investigations in some common cucurbits with special reference to fragmentation of chromosomes as a physical basis of speciation. *Genet.* **48:** 237-56.

Choudhury, B., and M. R. Thakur. 1965. Inheritance of sex forms in *Luffa*. *Indian J. Genet.* **25:** 188-197.

Clausen, J. 1961. Introgression facilitated by apomixis in polyploid poas. *Euphytica.* **10:** 87-94.

Dutt, B., and R. P. Roy. 1971. Cytogenetic investigations in Cucurbitaceae. I. Interspecific hybridization in *Luffa*. *Genetica* **42:** **139–156.**

Fryxell, P. A. 1957. Mode of reproduction in higher plants. *Bot. Rev.* **23:** 135-233.

George, Jr. William L. 1970. Dioecism in cucumber (*Cucumis sativa* L.). *Genetics* **64:** 23-28.

Grant, V. 1949. Pollination systems as isolating mechanisms in angiosperms. *Evolution* **3:** 82-97.

——. 1956. The influence of breeding habit on the outcome of natural hybridization in plants. *Am. Nat.* **90:** 319-322.

——. 1958. The regulation of recombination in plants. *Cold Spr. Harb. Symp.* **23:** 337-63.

——. 1965. Evidence for the selective origin of incompatibility barrier in the leafy-stemmed *Gilias. Proc. natn. Acad. Sci.* **54:** 1567-1571.

Harlan, J. R. 1963. Natural introgression between *Bothriochloa ischaemum* and *B. intermedia* in West Pakistan. *Bot. Gaz.* **124:** 294-300.

Hurd, P. D. Jr., and E. G. Linsley. 1964. The squash and gourd bees-genera *Peponapsis* Robertson and *Xenoglossa* Smith-inhabiting America north of Mexico (Hymenoptera: Apoidea). *Hilgardia* **35 :** 375-477.

Hutchinson, J. B. 1965. *Essays on Crop Plant Evolution.*

Kumar, L. S. S., and. G. B. Deodikar. 1940. Sex chromosomes of *Coccinia indica* Wight & Arn. *Curr. Sci.* **9:** 128-30.

——, and Vishveshwaraiah, S. 1952. Sex mechanism in *Coccinia indica*. *Nature, Lond.* **170:** 330-31.

Lewis, D., and L. K. Crowe. 1956. The genetics and evolution of gynodioecy. *Evolution* **10:** 115-125.

Mather, K. 1956. Polygenic mutation and variation in populations. *Proc. R. Soc., B.* **145:** 293-297.

Mayr, E. 1963. *Animal Species and Evolution.* Harvard Univ. Press, Cambridge.

Patel, G. I. 1952. Chromosome basis of dioecism in *Trichosanthes dioica*. *Curr. Sci* **21:** 343-44.

Richharia, R. H. 1948. Sex inheritance in *Luffa acutangula*. *Curr. Sci.*, **17:** 359.

Roy, R. P., and P. M. Roy 1970. Mechanism of sex determination in *Coccinia indica*. *J. Indian Bot. Soc.* Gol. Jub Vol. (in press).

Shifriss, O. 1961. Sex control in Cucumbers. *J. Hered* **52:** 5-12.

——, and E. Galun. 1956. Sex expression in the Cucumber. *Proc. Am. Soc. hort. Sci.* **67:** 479-486.

——, W. L. George, Jr., and J. A. Quinones. 1964. Gynodioecism in Cucumbers. *Genetics* **49**: 285-291.

Simmonds, N. W. 1962. Variability in crop plants, its use and conservation. *Biol. Rev.* **37**: 422-465.

Singh, H. B., S. Ramanujam, and B. P. Pal. 1948. Inheritance of sex forms in *Luffa acutangula* Roxb. *Nature, Lond.* **161**: 775.

Singh, R. N. 1953. Studies in the sex expression, sex ratio and floral abnormalities in the genus *Trichosanthes*. *Indian J. Hort.* **10**: 98-106.

Stebbins, G. L. 1950. *Variation and Evolution of Plants*. London.

——. 1957. Self fertilization and population variability in higher plants. *Am. Nat.* **91**: 337-354.

Tkachenko, N. N. 1935. Preliminary results of a genetic investigation of the Cucumber (*Cucumis sativus* L.) *Bull. Appl. Bot. Genet. Pl. Breed.* **9**: 311-356.

Thakur, M. R., and B. Choudhury 1966. Inheritance of some qualitative characters in *Luffa species*. *Indian J. Genet.* **26**: 79-86.

Trivedi, R. N., and R. P. Roy. 1972 Cytological Studies in some Species of *Momordica*. *Genet.* **43**: 100-109.

Westergaard, M. 1958. The mechanism of sex determination in dioecious flowering plants. *Adv. Genet.* **9**: 217-281.

Whitaker, T. W. 1956. Origin of the cultivated *Cucurbita*. *Amer. Natl.* **90**: 171-176.

——, and W. P. Bemis. 1964. Evolution in the genus *Cucurbita*. *Evolution* **18**: 553-559.

Adv. Pl. Morph. 1972: 203-210

GENETIC RECONSTRUCTION OF CROP IDEOTYPES

BY M. S. SWAMINATHAN

Indian Council of Agricultural Research New, Delhi-1, India

ABSTRACT

Tailoring the architecture of crop plants to suit specific production goals has now become possible through integrated research in plant physiology and genetics. Elegant genetic tools are available to breed strains possessing the desired ideotypes or conceptual plant types.

INTRODUCTION

The exploitation of heterosis in the form of commercial hybrids, the enhancement of the effects of additive gene action through synthetics and composites and the development of the concept of a dwarf plant type suited to good agronomy have all helped to raise the ceiling to yield in many crop plants to a level not considered possible before. Based on an understanding of the factors governing productivity, conceptual models or ideotypes (Donald, 1968) of crop varieties can be constructed, which if realised through breeding, would help to achieve still higher yields. The following are some of the desirable attributes of crop ideotypes, particularly in the tropics and subtropics where crop growth is possible throughout the year:

(a) Superior population performance.
(b) High productivity per day.
(c) High photosynthetic ability.
(d) Low photo-respiration.
(e) Photo and thermo-insensitivity.
(f) High response to nutrients.
(g) High productivity per unit of water.
(h) Multiple resistance to pests.
(i) Better protein quantity and quality.
(j) Crop canopies that can retain and fix a maximum of Co_2.

There are two basic groups of plants of importance in agriculture (Donald, 1968) a high photosynthetic capacity group including *Sorghum*, *Saccharum*, maize and some dicotyledons such as *Amaranthus and Atriplex* sp. and (Swaminathan, 1968) a lower capacity group including wheat, cotton, sugarbeet, pulses etc. Some of the characteristics of these two groups are given in Table I. One of the current aims of breeding research Is to examine the feasibility of converting a low capacity into a high capacity species.

It is such a shift in selection criteria that has provided special scope for deploying mutation breeding techniques very effectively in the development of crop varieties suited for present day needs (Swaminathan 1968, 1969). As a result, several new crop varieties developed by mutation breeding have been released in recent years for commercial cultivation in India (Table II). The results of some recent research at the Indian Agricultural Research Institute on the practical realisation of desirable ideotypes through mutation breeding are summarised in this paper:

BREEDING NEW IDEOTYPES OF WHEAT FOR UNIRRIGATED AREAS

Nearly 66% of the wheat area of about 14 million hectares in India depends for crop growth only on rainfall. Asana (1970) has developed an ideotype for such areas, which is designed to achieve a maximisation of the yield of the main tiller thereby compensating for poor tillering and upon the dissociation of undesirable associations such as earliness and poor root growth and a low number of spikelets per ear. This ideotype has the following attributes:

(a) A large number of spikelets and grains on the main ear through adventitious branching in the ear.

(b) A long peduncle which forms a substantial proportion of the length of the stem, thereby increasing photosynthesis in the peduncle and flag leaf.

(c) A semi-dwarf plant habit which helps to avoid lodging in fields supplied with fertilizer.

(d) About 7 leaves on the main axis with a large flag leaf; the leaves preferably being arranged horizontally so as to permit better retention of dew and also better light interception.

(e) A good growth of roots in the 3rd and 4th feet of soil depth.

(f) Emergence of ears at such a time that will permit grains to develop at least for 5 weeks at a mean maximum temperature of about 25°C.

A study was initiated by Mehta and Swaminathan (unpublished) in 1967 to ascertain the possibility of isolating genotypes with the above attributes. The material for the study consisted of an induced branched ear mutant of *Triticum aestivum* var. N P 797 (Swaminathan, et al. 1966) and tetraploid branched ear material derived from crosses between *T. durum* and *T. turgidum* var. mirabile. The material was treated with both gamma rays (20 Kr and 30 Kr), Ethylmethane Sulphonate (0.2%) and N-Nitroso-N-Methyl Urea (0.02%) Recurrent selection was practised for the desirable characters in M_2 and later generations. Selection was also done in progenies derived from crosses between branched ear types and other wheat varieties like Kalyan Sona and Sharbati Sonora. The extent of progress made so far in achieving the desirable ideotype is summarised in Table II. The data indicate that the chances for realising Asan's ideotype for unirrigated wheat are very high. The most promising selections

TABLE I

PHOTOSYNTHETIC SYSTEMS IN CROP PLANTS

Character	Low capacity species	High capacity species
Net photosynthetic rate in full sun, mg Co_2 day^{-2} hour^{-1}	25—45	45—60
Carbon path	Calvin cycle, fix Co_2 with C_3 acid	Hatch-Slack path, fix Co_2 with C_4 acid
Co_2 compensation point ppm V/V Co_2	>40	<10
Increase in net photosynthetic rate in low oxygen	Yes	No

are being subjected to a further cycle of mutagen treatment, selection and recombination.

and Jamuna (a dwarf, fine grain variety developed at slender grains) and Jamuna (a dwarf, fine-grain variety developed at

TABLE II

COMMERCIAL VARIETIES RELEASED IN INDIA THROUGH MUTATION BREEDING

Crop	Name of the variety		Mutagen and year of release.	Characteristics	Reference
	Parent	Mutant			
Wheat	N.P. 799	N.P. 836	X—rays, 1961	Fully awned and about 10% more yield.	Jagathesan et al. (1961)
	Sonora 64	Sharbati Sonora	UV + Gamma rays, 1967	Amber grain, high protein.	
Rice	T. 141	Jagannath	X—ray, 1969	Fertilizer responsive, short stature with an average height of 95 cms in contrast to 140 cm of T. 141.	S. K. Sinha (Unpublished)
Castor	H.C. 6.	Aruna	Thermal Neutrons 1968	120 days duration in contrast to 250 days in H.C. 6; high yield	Bala Narasaiah and Kulkarni (1969)
French Bean	Wax podded	Pusa Parvati	X—rays, 1969	Early, bush type with attractive light green pods.	Vishnu Swarup and Gill (1968)
Tomato	Sioux	S. 12	gamma rays, 1969	Dwarf, 30% more yield than Sioux	K. S. Nandpuri (Unpublished)
	Meeruti	Pusa Lal Meeruti	X—rays, 1969	Uniform red colour	Jain, et al. (1962)

ALTERING LEAF CHARACTERS IN RICE TO PROMOTE BETTER LIGHT INTERCEPTION

The introduction of the dwarfing gene in *Oryza sativa* sub sp. *indica* has helped to increase the yield of *indica* rices substantially through fertilizer application. In many high-yielding dwarf *indicas*, the total biological yield does not appear to be higher than in the tall *indicas*. The higher grain yield really arises from a higher harvest index (i. e., grain/straw ratio). The data from a study conducted by Asana and Salunke (unpublished) in Taichung Native 1 (a dwarf *indica* from Taiwan), Basmati 370 (a tall *indica* from India with long slender grains) the IARI by crossing Taichung Native 1 with Basmati 370 followed by four back-crosses to the Basmati parent) are given in Table III. A detailed analysis of the production physiological processes in these 3 varieties by Asana and Salunke indicated that the net assimilation rate decreases with a drop in light transmission about 30 days after transplanting. The rapid fall in light intensity after 30 days appeared to affect adversely tillering and the realisation of the full sink capacity. A smaller leaf size and a lower number of leaves hence appeared to be desirable attributes for promoting a higher net assimilation in rate thepopulation as a whole.

In order to examine the influence of leaf characters such as number, size, orientation and senescence pattern on percentage light transmission and net assimilation rate, the rice varieties Taichung Native 1 and Tainan 3 were treated with a wide range of mutagens including gamma rays, ethylmethane sulphonate and nitrosomethyl urea. Mutants with altered leaf characteristic were isolated in M_2, M_3 and later generations (Siddiq, Singh and Swaminathan, unpublished). There was considerable variation in the size, number, alignment and ageing pattern of leaves in the mutants. Some of these mutants had associated changes in panicle characters, such as density and fertility. Mutants with normal panicles but altered leaf characters are currently being developed for studies of light transmission and dry matter production.

TABLE III

PROGRESS MADE IN THE DEVELOPMENT OF A BRANCHED-EAR WHEAT STRAIN

Plant part	Characteristics of the initial material in 1967	Characteristics of selections
Ear	Erratic branching, low spikelet fertility and low 1000-kernel weight	Good branching with as many as 19 adventitious branches per spike, good fertility with as many as 207 grains per spike, good 1000-kernel weight.
Peduncle	Poor peduncle exertion	Peduncle index high, promoting greater photosynthesis in the peduncle.
Plant height	Semi-dwarf to dwarf (less than 100 cms).	Semi-dwarf to dwarf (less than 100 cms.)
Leaves	Leafy types with 14 to 15 leaves on the main stem	Two single leaf and several reduced leaf number mutants were isolated.
Roots	Variable depths	Deep root system, reaching the 3rd and 4th feet of soil depth
Ear	Very late, taking about 117 to 120 days from sowing to earing	Early mutants taking about 105 days from sowing to earing were isolated

Qualset et al. (1970) have recently described a mutant in bread wheat which influences the structure of the crop canopy and thereby affords an apportunity to evaluate light interception and the physiological aspects of crop productivity. Evans and Dunston (1970) have concluded from a study of physiological changes in the evolution of yield in *Triticum* species that so far evolution has progressed through changes in grain and leaf size and in the proportion of a dry weight mobilized to the grain. They, however, feel that while photosynthetic rate has not limited the evolution of yield in wheat upto the present, it may well limit further evolution. The proceedings of a recent Symposium on "Physiological aspects of crop yield" (Eastin, Haskins, Sullivan, Van Bavel, 1969) (8) also make it clear that the next major break-through in increasing productivity may come from the development of plant types which possess an inherently greater potential for biological yield, since the advantage conferred by alterations in the harvest index has already been exploited to a great extent.

INDUCTION OF MUTATIONS AT THE HAPLOID LEVEL

With the standardisation of a technique for the raising of haploids from anther cultures by Guha and Maheshwari (1964), a way is now open for the large scale production of haploids and their use in mutation breeding. While the original finding was made in *Datura innoxia*, subsequent research has shown that haploids of *Nicotiana tobacum* (Nakata and Tanaka 1968. Tanaka and Nakata 1969; Nitsch and Nitsch 1969; Sunderland and Wick, 1969) and of *Oryza sativa* (Nizeki and One, 1968; Guha et al. 1970) can be produced by in vitro anther culture. Nitsch and Pereau-Leroy (1969) have used mutagens in culture media for inducing mutations at the haploid level in *Nicotiana tabacum*. Gamma rays were more effective than EMS in yielding mutants (Nitsch, 1969).

For using this technique successfully, it is necessary to standardise techniques for getting consistently a large number of haploids. Since genotypic differences in the ability of pollen to form embryoids seem to exist, Guha, and Swaminathan, (unpublished) carried out a study with 20 different rice varieties. Since the techniques used may be of interest they are described in detail here.

Florets of twenty different varieties of rice were collected while still enclosed within the sheath. After sterilization in dilute chlorox solution, they were transferred to an inoculation chamber where the buds were dissected and five anthers were planted on a sterile nutrient medium. Blaydes' medium (Blaydes, 1966) for soybean callus cultures with 3% sucrose was used as the basal medium. Various growth substances such as indolacetic acid (IAA), 2. 4-dichlorophenoxyacetic acid (2, 4-D), Kinetin, yeast extract and coconut milk were also added to the basal medium, when required. The media were sterilized autoclaving at 15 lbs/in^2 for 15 minutes and solidified with 0.8% agar. The pH was adjusted to 5.8. Cultures were kept at 25°, for the initial 3-4 weeks in dark and then in light (warm white fluorescent, 1000 ft-c).

Anthers were planted at three stages of development—late meiotic and tetrad formation stage, uninucleate stage and on the day of anthesis. Young anthers implanted at the time of tetrad formation or earlier failed to grow on any media. When planted on the day of anthesis, when pollen grains are mature, most of the anthers opened liberating the pollen grains which germinated giving rise to pollen tubes. Only anthers containing uninucleate pollen grains showed differentiation of pollen embryoids. Therefore, further experiments were carried out only with anthers containing uninucleate pollen grains. These results are similar to those reported for tobacco pollen.

In the basal medium without any growth adjuvants, anthers degenerated two to three weeks after inoculation. The addition of coconut milk (15%) and yeast extract (1000 ppm) stimulated many pollen grains to divide. The incorporation of 2 ppm IAA and 2 ppm 2.4-D and 1 ppm kinetin further stimulated division and, therefore, this medium was generally used for the induction of mitosis in pollen grains.

Acetocarmine squashes of the anthers were prepared to regular intervals throughout the culture period to assess the state of development of pollen. After one week in culture, some of the pollen grains started enlarging and attained two to three times their original volume. In these grains, the cytoplasm stained very densely. In another week, they underwent mitosis and binucleate, where both the cells were morphologically alike. Soon, mitosis commenced in both the cells, which continued giving rise to multicellular pollen grains 3-4 weeks after planting. In older cultures, these multicellular masses (embryoids) burst out of their original wall and assumed globular stage. If embryoids were dissected out at this stage and planted on a fresh medium containing coconut milk (15%) without auxin, they exhibited organized growth and developed into normal plantlets. Chromosome counts in root tips in several such seedlings revealed that they are haploids with 2n=12. However, if embryoids were allowed to grow in the original medium where they arose, divisions in the pollen grains continued in an irregular manner resulting in a mass of callus. It appeared that during the initial stages of developement, 'pollen embryoids' get attached to the wall of the anthers by a suspensor-like outgrowth. It is very easy to miss this attachment, if anthers are squashesd or subjected to even slight pressure. Careful dissections revealed this attachment, the anther wall apparently acting like a placentum. However, after some time, the embryoids get detached from the anther wall and grow independently.

There was a wide variation in the twenty varieties studied with regard to their potential for giving rise to androgenic haploids through this technique. Some primitive cultivars from Assam like Assam-5 gave an excellent response. Tropical *japonic* varieties from Taiwan like Taichung 65 and Tainan-3 did not behave any differently than many of the *indica* varieties. The dwarf, high yeilding *idica* strains IR-8, developed at the International Rice Research Institute, the Philippines, was found suitable for use in such studies. The promising varieties are now being used in studies on the in-

TABLE IV

YIELD CHARACTERISTICS IN DWARF AND TALL *INDICA* RICE VARIETIES

Character	VARIETY		
	Taichung Native-1	Basmati-370	Jamuna
FIELD			
Grain yield (Q/ha)	74.4	31.1	52.8
Straw yield (Q/ha)	73.3	121.0	63.7
Total biological yield (Q/ha)	147.7	152.1	116.5
POT			
Grain yield (gm/plant)	91.0	72.5	84.7
Straw yield (gm/plant)	73.4	116.4	91.2
Harvest index	55.4	38.4	48.2

duction of mutations at the haploid level. This technique is likely to become very important in plant breeding, particularly in perennial plants.

TABLE V

VARIATION IN EMBRYOID FORMATION IN RICE VARIETIES

Sl. No.	Strain	No. of anthers inoculated	Response
Indica strains			
1.	Assam 271	160	Some callusing of pollen grain, but no embryoid formation.
2.	Assam 10456	130	No response
3.	Assam 10303	80	Some callusing of the pollen but no embryoid formation.
4.	Assam Local	100	No response
5.	Assam 5955	1735	11% anthers produced pollen embryoid
6.	Assam 5	172	26% anthers produced pollen embryoid
7.	Tripura 2–5744	186	No response
8.	Assam culture	100	No response
9.	Soft grain type of West Bengal	245	Callusing
10.	Sabarmati	230	No response
11.	TR–8	1576	0.8% of the anthers showed pollen embryoids.
12.	IR–532	100	No response.
13.	Taichung Native—1	360	Pollen grains enlarged but no division.
14.	N. P. 130	600	No response.
15.	Basmati–370	1735	0.3% anthers produced pollen embryoids.
16.	Kala-Basmati	20	No response
17.	Sigadis	100	No response
18.	Sigadis IR–8 hybrid	148	No response
Japonica strains			
19.	Taichung 65	720	Slight callusing but no embryoid formation.
20.	Tainan 3	480	No response

REFERENCES

Asana R.D. 1970. The design of wheat ideotype. In *A New Technology for Dry Land Farming*. Indian Agricultural Research Institute, Delhi.

Bala Narasaiah D., and L.G. Kulkarni. 1969. Castor NPH-1 scores over HO-6. *Indian Farming* **18**: 11.

Blaydes, D.F. 1966. Interaction of kinetin and various inhibitors in the growth of soybean tissue. *Physiologia Pl.* **19**: 48.

Donald, C. M. 1968. The design of a wheat ideotype. *Proc. 3rd Intern. Wheat Genetics Symp., Canberra* 377.

Eastin, J. D., F. A. Haskins, C. Y. Sullivan, and C.P.C.H.M. Van Bavel. 1969. *Physiological Aspects of Crop Yield.* American Society of Agronomy.

Evans L. T., and R. L. Dunstone. 1970. Some physiological aspects of evolution in wheat. *Aust J. Biol. Sci.* **23**: 725.

Guha, S., R. D. Iyer, N. Gupta, and M. S. Swaminathan. 1970. Totipotency of genetic cells and production of haploids in rice. *Curr. Sci.* **89**: 171.

—, and S. C. Maheshwari. 1964. *In vitro* production of embryos from anthers of *Datura*. *Nature, Lond.* **204**: 497.

Jagathesan, D., C. H. Bhatia, and M. S. Swaminathan. 1961. Effect of induced awn mutations on yield in wheat. *Nature, Lond.* **190**: 468.

Jain, H. K., S. C. Sur, and R. N. Raut. 1962. Genetic studies in tomato. I-Induced variability for uniform fruit ripening and other characters. *Indian. J. Genet.* **22**: 81.

Nakata, K., and M. Tanaka. 1968. Differentiation of embryoids from developing germ cells in anther culture of tobacco. *Jap. J. Genet.* **44**: 88.

Nizeki, H., and K. O. One. 1968. Induction of haploid rice plant from anther culture. *Proc. Japan Acad.* **44**: 554.

Nitsch, J. P. 1969. Experimental androgenesis in *Nicotiana*. *Phytomorphology* **19**: 389.

—, and C. Nitcsch. 1969. Haploid from pollen grains. *Science* **163**: 85.

—, Pereau-Leroy. 1969. Obtention de mutants a partir de Nicotiana haploides issues de grain de pollen *C.r. hebd. seanc. Acad. Sci., Paris.* **269 D**: 1650.

Qualset, C. O., G. N. Ficle, M. K. Constantin and T.S. Osborne. 1970. Mutation in length affects wheat plant type. *Science*. **169: 1090.**

Sunderland, N., and F. M. Wicks. 1969. Cultivation of haploid plants from tobacco pollen. *Nature, Lond.* **224**: 1227.

Swaminathan, M. S. 1968. Mutation breeding. *Proc, XII. Intern. Congr Genet.* **3**: 327.

—.1969. Role of mutation breeding in a changing agriculture. In *Proc. Symp on Induced Mutations in Plants*. IAEA, Pullman. 719.

— , V. L. Chopra and G. R. K. Sastry, 1966. Expression and stability of an induced mutation for ear branching in bread wheat. *Curr. Sci.* **35**: 91.

Tanaka, M., and K. Nakata. 1969. Tobacco plants obtained by anther culture and the experiment to get diploid deeds from haploids *Jap. J. Genet,* **44**: 47.

Vishu Swarup, and H. S. Gill. 1968. X-ray induced mutations in French Benan. *Indian J. Genet.* **28**: 44.

Adv. Pl. Morph. 1972 : 211-217.

SOME ASPECTS OF THE FLORAL MORPHOLOGY AND EMBRYOLOGY OF *FLAGELLARIA INDICA* LINN.

BY K. SUBRAMANYAM AND H. S. NARAYANA

Botanical Survey of India, Calcutta and Department of Botany, University of Rajasthan, Jaipur, India

ABSTRACT

The flower of *Flagellaria indica* is sessile, bracteate, bisexual, pentacyclic, trimerous and hypogynous. The sepals, petals, outer and inner whorls of stamens and the carpels are arranged in regular alternate spirals of clockwise and anticlockwise sequence. The gynoecium is tricarpellary and trilocular with one pendulous, orthotropous and crassinucellate ovule in each locule borne on an axile placenta. The sepals, petals and stamens receive a trace each. The entire placental vascular tissue is used up in the supply of the ovules. The wall of the tetrasporangiate anther consists of a secretory tapetum, two middle layers and a fibrous endothecium beneath the epidermis. The 1-ulcerate pollen grains are shed at the 2-celled stage. The development of the embryo sac follows the Allium type. The vesicular egg is situated between the flask-shaped synergids which show a filiform apparatus. The upper polar nucleus is larger than the lower and the antipodal cells are ephemeral.

INTRODUCTION

Flagelleriaceae are a monocotyledonous family confined to the warmer parts of the world and include three genera. On a wide range of evidence the assemblage is unnatural according to Tomlinson (1969). There is meagre information on the various aspects of the floral morphology of the family (Davis, 1966). The present paper deals with *Flagellaria indica* Linn. a climbing shrub, which exhibits true dichotomy of its branches (Tomlinson, 1970), a rare phenomenon among angiosperms. Palynology of this species was described by Chanda (1966). Chuang et al. (1963), Shetty and Subramanyam (1964), and Briggs (1966) reported the chromosome number of the species as 2n=38 which is also the number for other species of *Flagellaria* (Mangenot and Mangenot, 1958, 1962). Shetty and Subramanyam (1964) reported simultaneous division of microsporocytes which is not very common among monocotyledons. They also observed giant pollen grains and some of them were constricted in the middle. The vegetative anatomy of *Flagellaria* has been described by Tomlinson (1969).

MATERIALS AND METHODS

Materials used in the present investigation were collected and fixed by Mr V. Abraham, from Changail (Howrah Dist.) in 1965. Voucher specimen No. 269 of this material is deposited in the

---

We are thankful to Dr N. C. Nair for a critical reading of the manuscript and Mr V. Abraham for the help rendered in the collection of the material.

herbarium of the Botanical Survey of India, Calcutta (Cal). Customary methods were followed for fixation and sections were cut at a thickness of 10 to 14 microns. The usual procedure for staining was followed.

MORPHOLOGY OF THE FLOWER

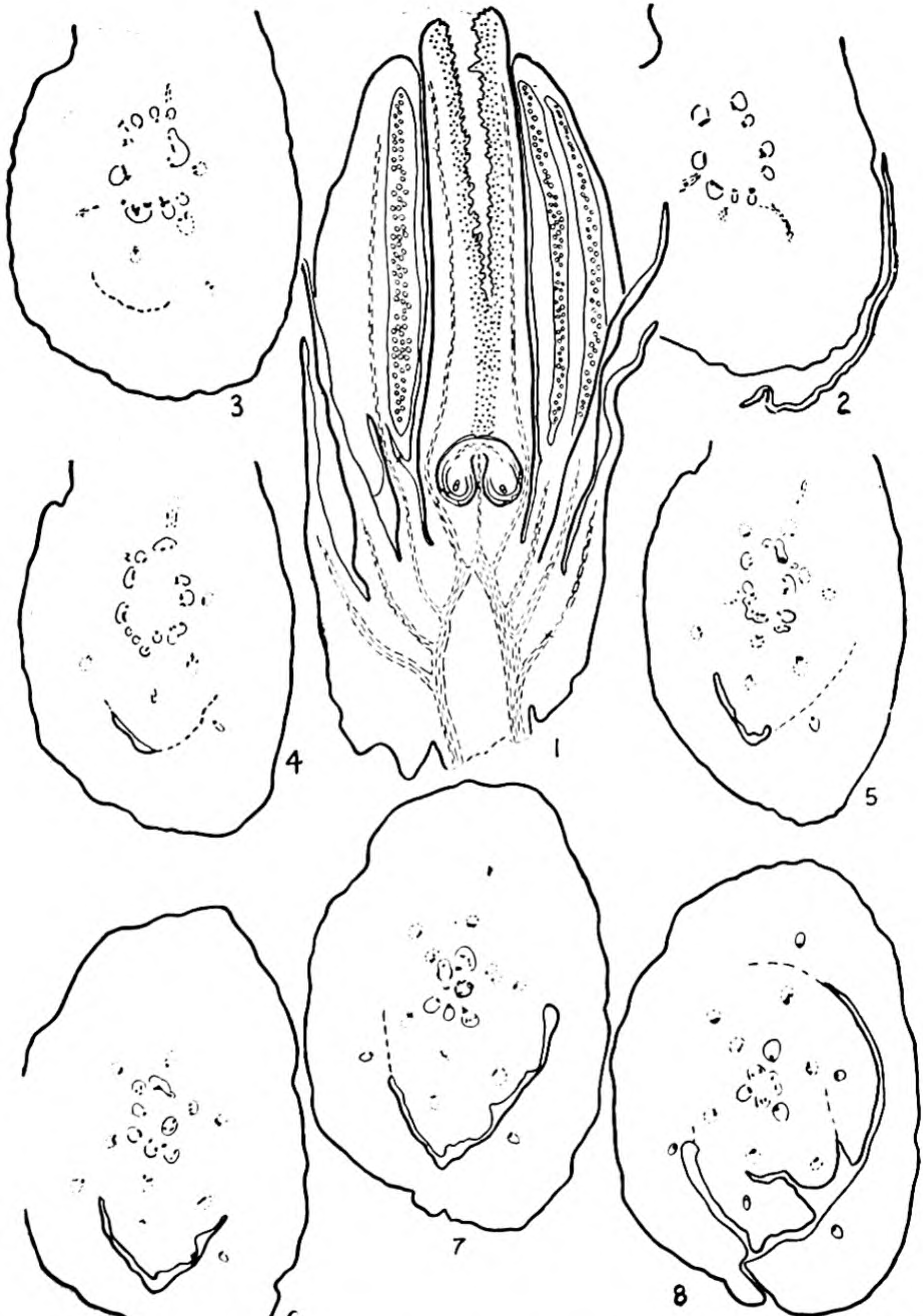
The flowers are sessile, bracteate, ebracteolate, bisexual, pentacyclic, trimerous and hypogynous. There are six stamens almost equalling the styles and arranged in two whorls. The anthers are dithecous, basifixed, latrorse and tetrasporangiate. The gynoecium is tricarpellary, syncarpous and trilocular (Fig. 11) with one median pendulous ovule in each locule borne on an axile placenta. The style is split into three branches. Occasionally stamens and carpels show reduction in their number: thus only five or four stamens (Figs. 15, 16) and even two carpels were observed.

A ring of collateral vascular bundles enter the flower. Traces for the sepals depart from this ring in a clockwise direction anterioposteriorly. Each sepal receives only one trace (Fig. 2) which continues without branching. The sepal traces are followed by petal traces in an anticlockwise sequence anterioposteriorly (Figs. 2, 3). Traces to the antisepalous and antipetalous stamens depart anterioposteriorly in a clockwise and anticlockwise sequence respectively (Figs. 4, 7). The dorsal traces of the three carpels are demarcated next in a clockwise direction. Six bundles which are left in the centre now function as the ventral traces of the carpels (Fig. 8). The ventral bundles of adjacent carpels fuse in radii alternating with the dorsal bundles of the carpels (Fig. 9), and all the three bundles thus formed again fuse to form a single placental strand in the centre (Fig. 10) which in turn traverses almost the entire length of the placenta where it breaks up finally at its tip into three ovular traces to the respective carpels (Fig. 12). The dorsal bundles continue in the style and each of them enters a branch. The inner side of the stylar branches is covered with stigmatic papillae (Figs. 1, 14).

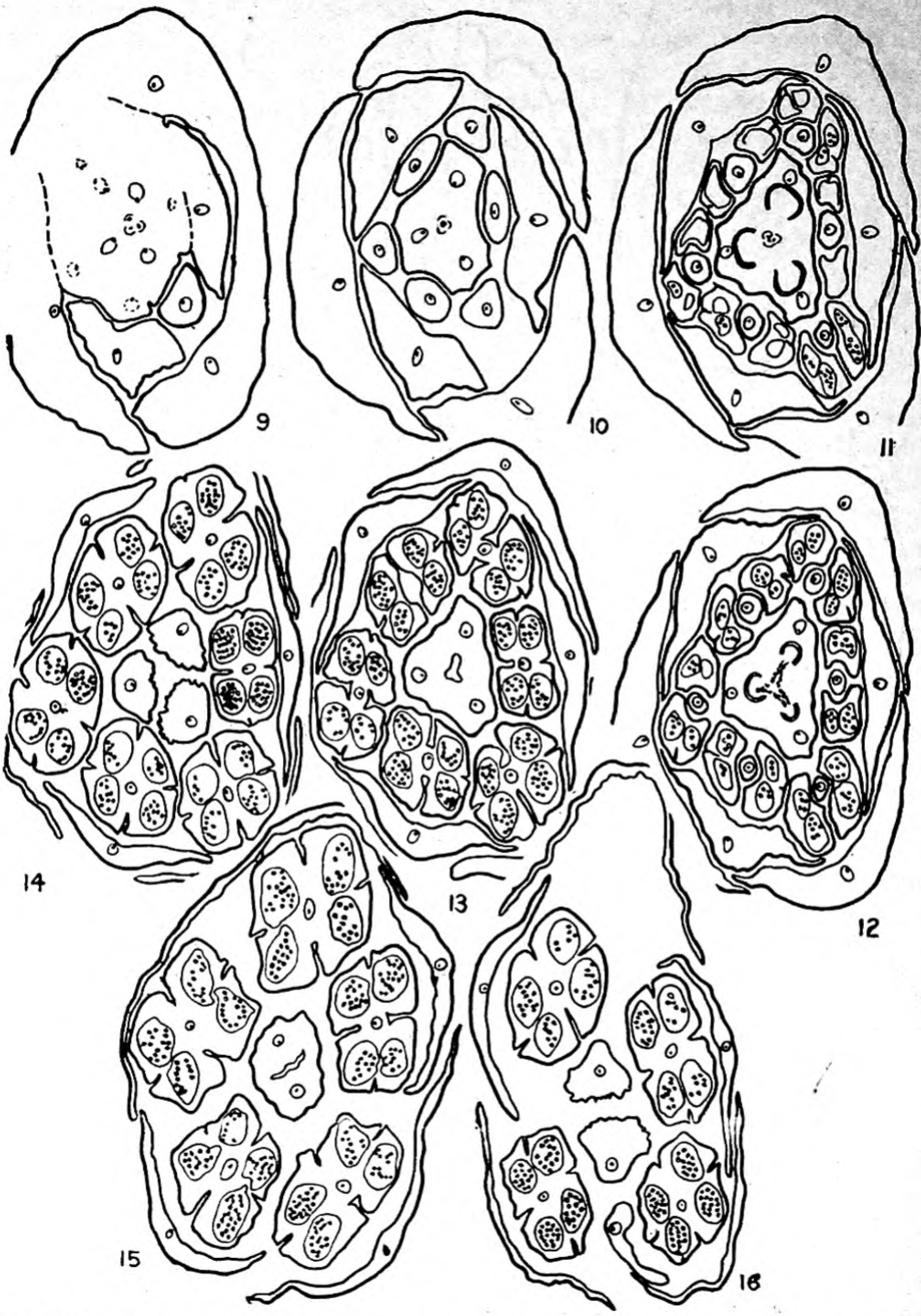
MICROSPORANGIUM, MICROSPOROGENESIS AND MALE GAMETOPHYTE

The anthers are tetrasporangiate. However, an instance of a trisporangiate anther with one vascular bundle (Fig. 17) and hexa to hepta-sporangiate anthers with two vascular bundles were observed (Figs. 18, 19). The trisporangiate anther suggests the merging of two of the four microsporangia into one while the hexa- and hepta-sporangiate anthers indicate that they are formed by the fusion of two stamens and reduction in the number of microsporangia.

The male archesporium is hypodermal. It divides periclinally to form an outer primary parietal layer and an inner primary sporogenous layer. The primary parietal layer divides anticlinally and periclinally to form an anther wall of four layers besides the epidermis (Figs. 20, 21). The inner sporogenous layer divides to form a large number of microsporocytes. The rectangular cells of the endothecium are larger than the epidermal cells (Figs. 22, 23). They develop fibrous thickenings in a few cells (Fig.23). The middle layer is one to two cells thick. It is tangentially compressed during the microspore formation and totally obliterated in the median plane of the microsporangium. The tapetum is unilayered except at certain regions where it is more



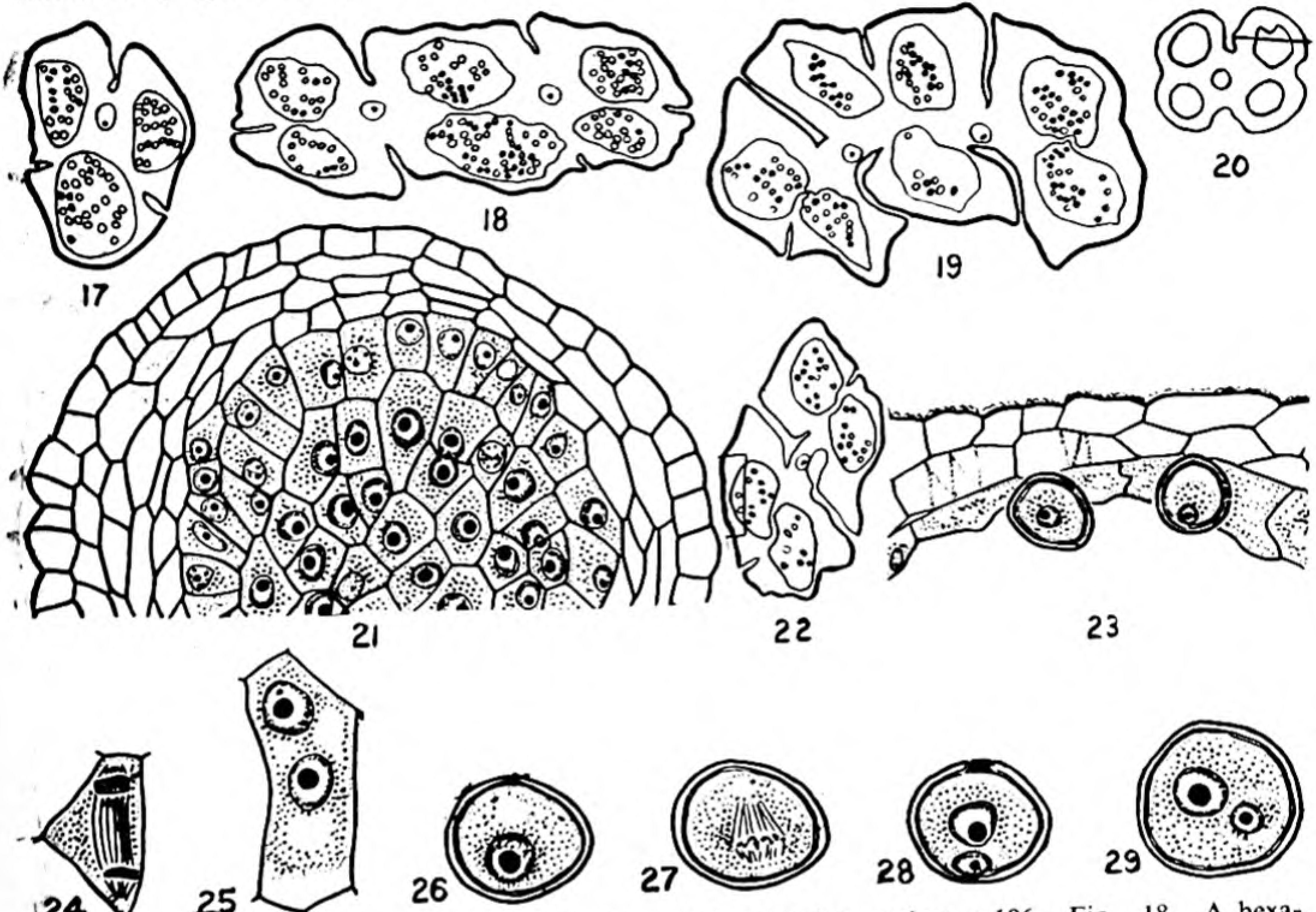
FIGS. 1-8. *Flagellaria indica.* Fig. 1. L. s. flower bud, x 191. Figs. 2-8. Transections of flower bud at different levels from base to apex, x 106.


9
10
11
14
13
12
15
16

FIGS. 9-16. *Flagellaria indica.* Transections of flower bud at different levels from base to apex, x 106.

than one cell thick (Fig. 21). The cells are radially elongated and one or two nucleate (Figs. 24, 25). As the anther matures the tapetum becomes emaciated (Fig. 23) and ultimately disappears. In the mature anther the epidermal cells are cutinized (Fig. 23).

The microspores are spherical in cross section. The spore wall differentiates into the outer thick exine and the inner thin intine. The microspore nucleus moves towards the wall (Fig. 26) where it divides unequally (Fig. 27) forming a vegetative and a generative cell (Fig. 29). The generative cell is small and lenticular and has an ellipsoidal nucleus to start with which in turn later becomes spherical. The 1-ulcerate pollen grain is shed at the 2-celled stage (Fig. 29).

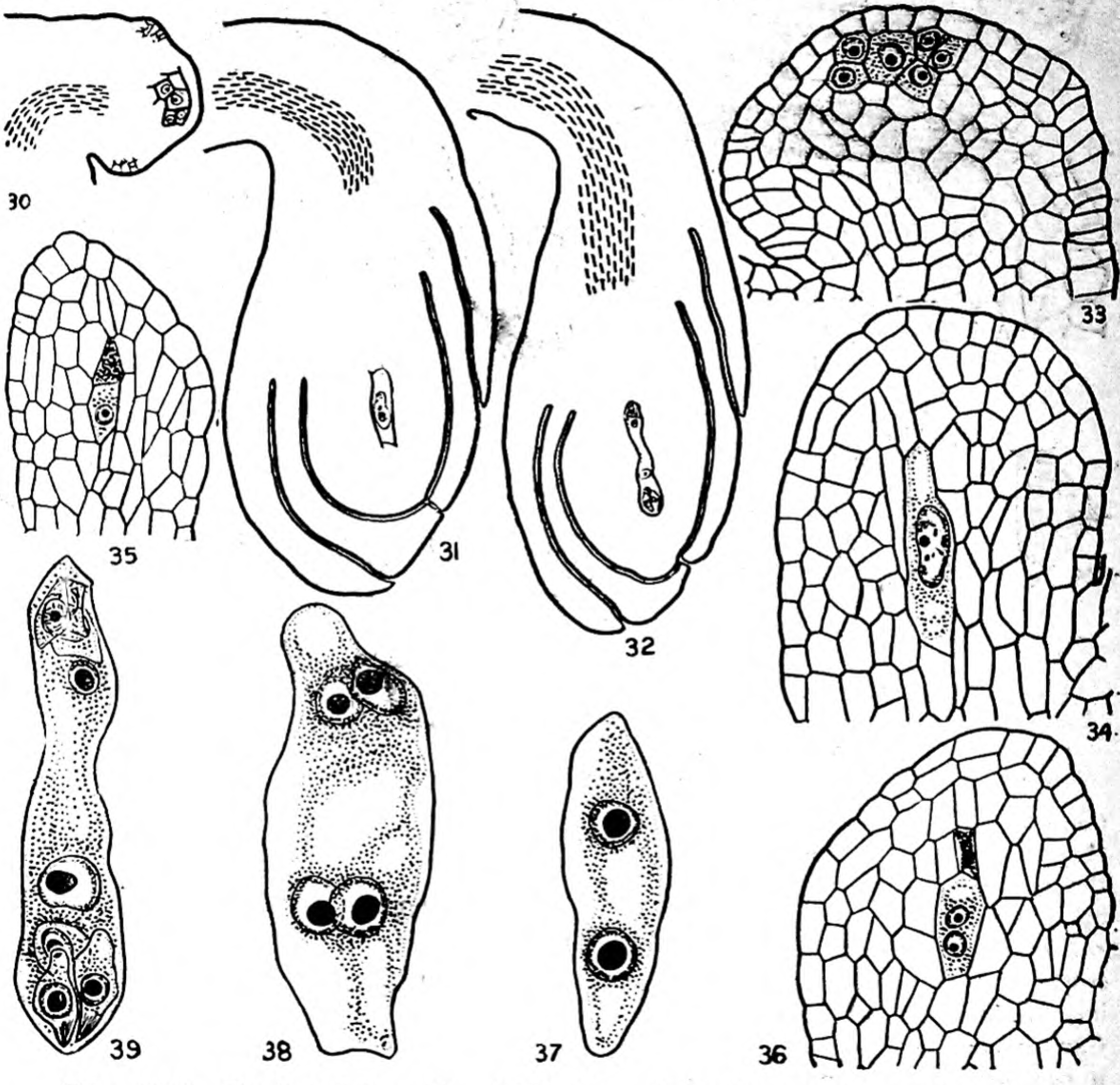


FIGS. 17-29. *Flagellaria indica*. Fig. 17. A trisporangiate anther, x 106. Fig. 18. A hexasporangiate anther, x 106. Fig. 19. A heptasporangiate anther, x 106. Fig. 20. Outline of T. s. anther at microsporocyte stage, x 106. Fig. 21. Magnified view of portion of microsporangium marked in Fig. 20, x 1000. Fig. 22. Outline of T. s. anther at the microspore stage, x 106. Fig. 23. Magnified view of portion marked in Fig. 22, x 1000. Fig. 24. Tapetal cell in division, x 1933. Fig. 25. Bi-nucleate tapetal cell, x 1933. Fig. 26. Uninucleate microspore, x 1933. Fig. 27. Microspore nucleus in division, x 1933. Figs. 28, 29. 2-celled pollen grains, x 1933.

OVULE, MEGASPOROGENESIS AND FEMALE GAMETOPHYTE

The ovule develops in the form of papillate outgrowth on the placenta. It grows in length and expands at the tip (Fig. 30) before the division of the female archesporium. The integumentary initials differentiate simultaneously with the formation of the megasporocyte. The inner integument grows faster than the outer integument and organises the endostome when the megasporocyte is deep-seated (Fig. 31). The outer integument remains far behind the endostome forming a collar. The ovule is pendulous, bitegmic, crassinucellar and nearly orthotropous since the micropyle, nucellus and hilum are almost in a straight line (Figs. 31, 32).

The hypodermal archesporium is multicellular. They divide to form the primary parietal cells and megasporocytes (Fig. 33). Ultimately only one megasporocyte is functional. The primary parietal cell does not divide again but radially elongates. The epidermal cells at the micropylar end divide



FIGS. 30-39. *Flagellaria indica.* Figs. 30-32. Stages in development of orthotropous ovule, x 433. Fig. 33. L. s. nucellus showing hypodermal multicellular archesporium; a few cells have already divided, x 1000. Fig. 34. Megasporocyte, x 1000. Fig. 35. Dyad, the upper cell degenerated, x 1000. Fig. 36. Another dyad in which the lower is 2-nucleate and upper degenerated, x 1000. Fig. 37. 2-nucleate embryo sac, x 1933. Fig. 38. 4-nucleate embryo sac, x 1933. Fig. 39. Mature embryo sac, x 1933.

periclinally as a result of which the megasporocyte becomes deep-seated. Its nucleus now enlarges, occupies the central position of the cell, (Fig. 34), and divides to form the cells of the dyad. The upper dyad cell degenerates (Fig. 35) and persists up to the 2-nucleate stage of the embryo sac (Fig. 36), while the lower dyad cell functions (Figs. 35, 36). The nucleus in the lower dyad cell divides to form two nuclei which move to the opposite poles and a large vacuole appears at the centre (Fig. 37). Further divisions result in the 4- (Fig. 38) and 8-nucleate stages of the embryo sac. Thus the development of the embryo sac conforms to the Allium type (Maheshwari, 1950).

In the mature embryo sac (Fig. 39), the cells of the egg apparatus are larger than the antipodal cells. The synergids are flask-shaped with a vacuole below a centrally situated nucleus and a filiform apparatus above. The egg is small, vesicular and situated between the synergids. The polar nuclei increase in size and are larger than the remaining nuclei in the embryo sac. Of the two polar nuclei, the upper polar nucleus is larger than the lower polar nucleus and is closer to the egg apparatus (Fig. 39). The lower polar nucleus migrates upward where it fuses with the upper polar nucleus to form the secondary nucleus. The antipodal cells are ephemeral.

REFERENCES

BRIGGS, B. G. 1966. Chromosome numbers of some Australian monocotyledons. *Contr. N. S. W. natl. Herb.* **4**: 24-34.

CHANDA, S. 1966. On the pollen morphology of the Flagellariaceae with reference to taxonomy. *Trans. Bose Res. Inst.* **8**: 53-55.

CHUANG, T. I., C. Y. CHAO, W. W. L. HI, AND S. C. KWAN. 1963. Chromosome numbers of the vascular plants of Taiwan I. *Taiwania* **1**: 51-66.

DAVIS, G. L. 1966. *Systematic Embryology of Angiosperms.* New York.

MAHESHWARI, P. 1950. *An Introduction to the Embryology of Angiosperms.* New York.

MANGENOT, S., AND G. MANGENOT. 1958. Deuxième liste de nombres chromosomiques nouveaux chez diverses Dicotyledones et Monocotyledones d' Afriqua, Occidentale. *Bull. Jord. bot. Etat Brux.* **28**: 315-329.

——, AND ——. 1962. Enquete sur les nombers chromosomiques dens une collection d' espèces tropicales. *Rev. Cyt.* et *Biol. Veg.* **25**: 411-447.

SHETTY, B. V., AND K. SUBRAMANYAM. 1964. Cytology of *Flagellaria indica* Linn. *Curr. Sci.* **33**: 279-280.

TOMLINSON, P. B. 1969. *Anatomy of Monocotyledons.* C.R. Metcalfe (ed.). III. *Commelinales to Zingiberales.* Oxford.

——. 1970. Dichotomous branching in *Flagellaria indica* (Monocotyledons). In *New Research in Plant Anatomy. Suppl. Bot. J. Linn. Soc. London* **63**: 1-14.

Adv. Pl. Morph. **1972: 218-232.**

STUDIES ON PALMS : EMBRYOLOGY OF *CARYOTA URENS* L.

BY NANDINI S. SHIRKE AND T. S. MAHABALE

Maharashatra Association for the Cultivation of Science, Poona-4, India

ABSTRACT

The paper gives an account of the embryology of *C. urens*. The inflorescences in the genus *Caryota* arise basipetally, which is an unusual feature in palms. They consist of primary and secondary axes. The primary axis is a stout peduncle protected by bracts with numerous secondary pendulous branches which bear fruits. They have both male and female flowers on each secondary branch. In *C. urens* there is one female flower between two male ones. The megasporogenesis is normal, giving rise to a 8-nucleate Polygonum type of embryo sac. The megaspore tetrads are linear. Microsporogenesis is regular and shows 18 bivalent chromosomes. Pollen grains are monocolpate and are shed in 2-celled condition. They germinate readily.

Development of the embryo is of the *Asterad* type. Endosperm is ruminated. Seeds germinate after about a year. A cotyledonary tube which morphologically is a hypocotyl comes out of the micropylar end and forms a frill around the carpellary eye on the endocarp. A small papilla is seen at its centre which is the root primordium. It grows considerably as a tube and carries down with it the apical meristem of the young seedling, ensheathed in the cotyledonary tube. The first foliage leaf developed from growing apex emerges from it by piercing it from a small oblique slit. The germination of seed is of the Remotive type.

Excised embryos of 30 days or more could grow well in cultures in White's basal medium or in Knop's solution. They developed much more rapidly than those developed in seeds in the soil.

INTRODUCTION

The genus *Caryota* is of Indo-Malayan origin. It has about a dozen valid species, of which four occur in India. *Caryota urens* L. is the commonest, being both wild and cultivated. The genus represents the sub-family Caryotoideae which is intermediate between the palmate-leaved palms and pinnate-leaved ones. It was, therefore, thought worthwhile investigating it morphologically as well as embryologically. Part I dealt with the former (see Mahabale and Shirke, 1968) and the present part deals with the embryology of *C. urens*.

Literature on the embryology of Palmae is rather scanty and needs reinvestigation in many species as remarked by Schnarf (1931). Quisumbing and Juliano (1927-1931) have described the development of ovule, embryo sac and male flowers in *Cocos nucifera*. Swamy (1942) has written a note on the megasporogenesis in *Areca catechu* and embryo development in it by C.V. Rao (1955). Selvaratnam (1952) described the embryo in Coconut and Kulkarni (1965) studied it further in three species of *Cocos*. De Poerck (1950) has reported *Adoxa* type of embryo sac in *Elaeis guineensis*, which later was found to be of the *Polygonum* type by Kajale and Ranade (1952, 1953). They also found four kinds of megaspore tetrads in that palm. Mahabale and Cheenaveeraiah (1957) next gave an account of the cytoembryology of *Hyphaene indica*, and C. V. Rao (1959a, b) in some members of the Ceroxylineae and Sabaleae. Mahabale (1966) has elucidated the structures observable in the morphology of pálm embryo, high lighting its early stages, special organs and later stages developed during germination. Mahabale and Biradar (1968) have studied the embryology of *Phoenix sylvestris* Roxb. Biradar (1968) made further studies on the embryology of *Phoenix pusilla* Gaertn., *P. acaulis* Buch. and *P. reclinata* Jacq. The present paper gives an account of the embryology of *C. urens* L. mainly, though a brief comparison of it with other species was given by Shirke (1966). These species are still under investigation. In one of them *C. rumphiana* Mart. polyembryony was noticed.

OBSERVATIONS

Inflorescence and flowers.—The inflorescence in this palm is a much branched spadix (Fig. 1), having numerous pendulous spikes arising as secondary branches from the top of a stout primary axis of the penduncle (Pl. I, Fig. 2; Fig. 1). Five to six such inflorescences can be seen at a time on the tree, developing basipetally. Each spadix is about 80 cm long, enclosed in a large spathe.

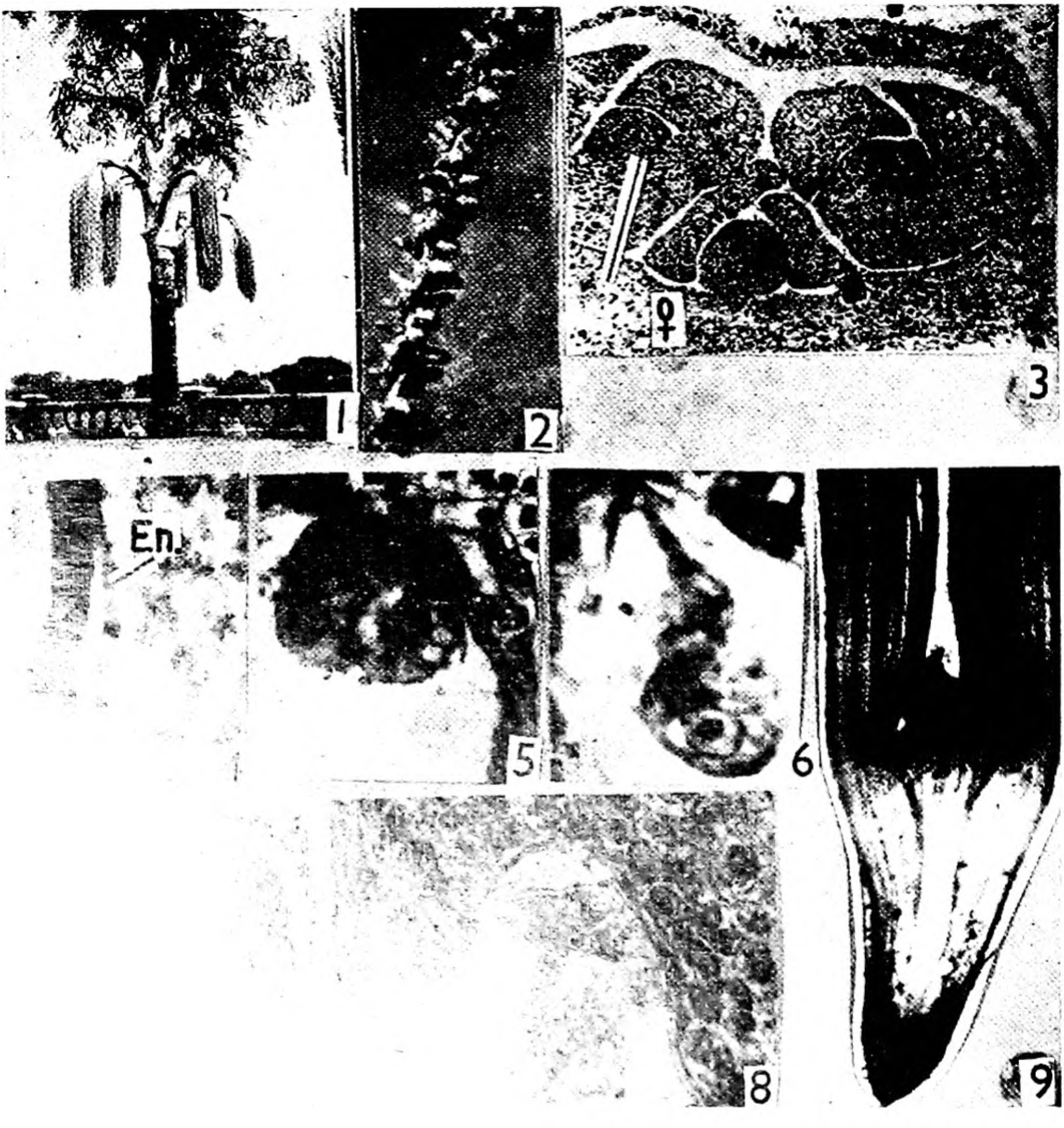
The flowers are unisexual and are grouped in threes, the central flower being female and the two side ones male (Pl. I, Fig. 3; Fig. 6). The male and female flowers have two perianth bracts, three sepals and three petals (Figs. 2, 3). There are 40 stamens in this species (Text Fig. 4). The female flowers have three filamentous staminodes (Fig. 5).

Organogeny.—The development of male flowers follows the same pattern as was seen by C. V. Rao (1959a) in this species. The stamens are differentiated into a filament and the anther lobes before the carpels are fully formed.

A female flower primordium arises in the axil of a bract. The outer perianth is about 8-layered. The inner perianth also develops with carpel primordium and is 2-3—layered. Air cavities, tannin containing cells, raphide-sacs and mucilage cells are common in the developing perianth of male as well as female flowers.

Microsporangium and *microsporogenesis*.—The archesporium in a developing anther is differentiated from one or two hypodermal cells (Fig. 7). The hypodermal initials form the primary parietal and primary sporogenous cells. By further divisions in the primary parietal cells a 5-layered wall of the anther is formed. Tapetum is of the secretory type and there is secondary increase in the sporogenous tissue up to 15 cells or more as could be seen in a T. s.

The process of meiosis is normal. At diakinesis 18 bivalents were counted in *C. urens* as were previously noted by Mahabale and Cheenaveeraiah (1957) (Fig. 17). Cytokinesis takes place by simultaneous cell plate formation. The microspore tetrads are generally tetrahedral, sometimes isobilateral (Figs. 9, 8). Pollen grains are bi-nucleate at the time of shedding (Figs. 10, 12, 15). They are monocolpate and have finely reticulate exine (Figs. 13, 16). They are monosiphonous, and the germ tube is twisted as in *Areca catechu* (Fig. 14).



[illegible] *ns* L. growing in the Municipal Garden at Hydera- [illegible] inflorescences. Fig. 2. Unopened male and female [illegible] ing spike showing a group of three flowers. The [illegible] deeply situated is female (♀), x 62. Fig. 4. L. s. of an ov- [illegible] patch of tannin-containing cells and the protoplasmic [illegible] the embryo sac, x 123. Fig. 5. Egg-like synergids and [illegible] embryo, x 166. Fig. 7. L. s. of an advanced stage in the [illegible] x 600. Fig. 8. Four-celled embryo. Note the persistent syn[illegible] cotyledonary tube at the first nodal point showing the develop- ing [illegible] developing shoot-apex, x 65.

En.: endosperm nuclei; [illegible] containing cells.

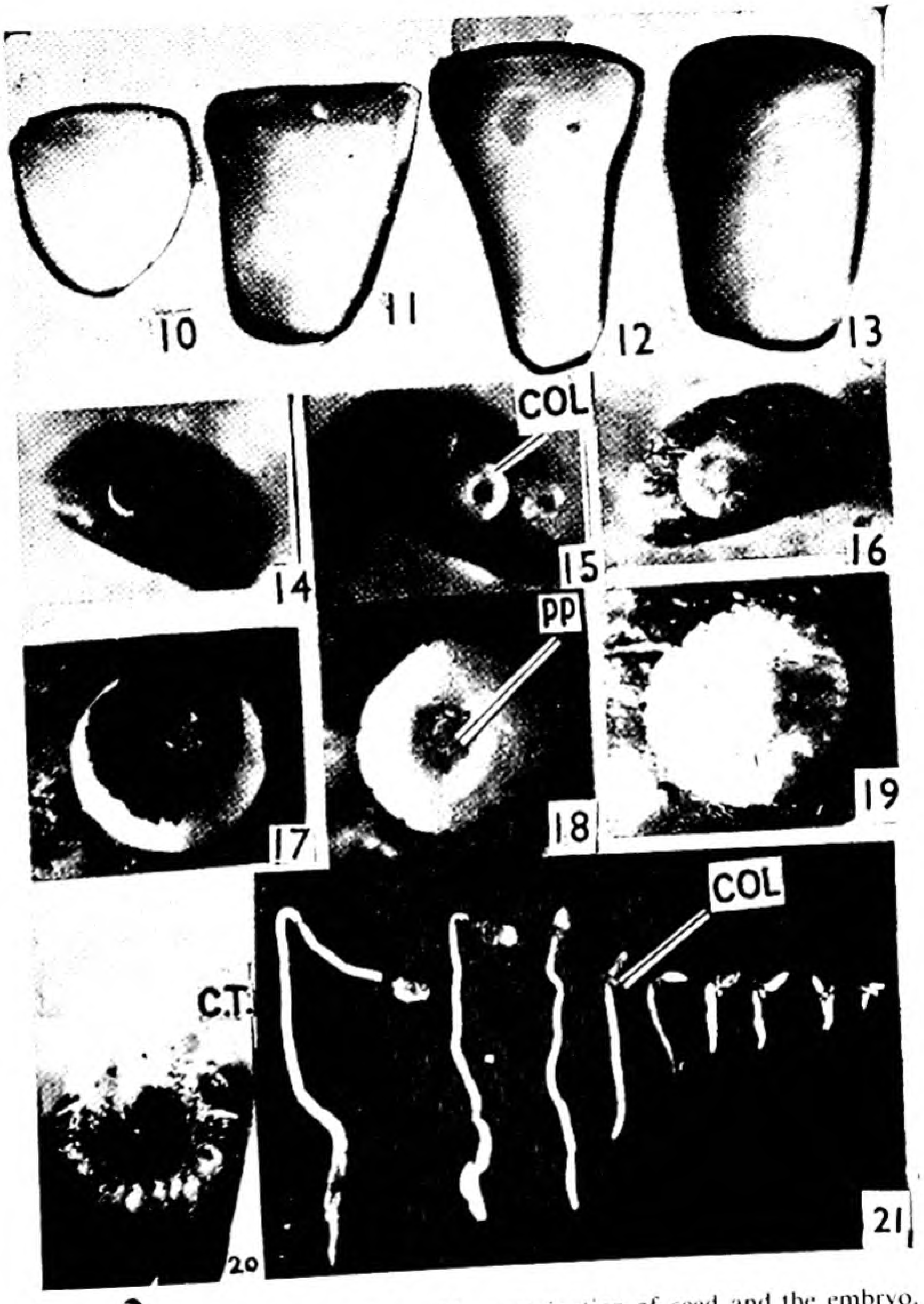


PLATE-II. FIGS. 10-21. *Caryota urens* L. The germination of seed and the embryo. Figs. 10-18. Embryos at different stages of growth dissected out from seeds during germination, × 24. Fig. 14. Seed showing a growing embryo emerged out of the endocarp, note that the collar with the seed coat is still adhering to endocarp, x 1·5. Fig. 15. Further growth of the collar, x 4. Fig. 16. Frilled edge of the collar and the root tip embedded in it, x 1·5. Figs. 17-19. Magnified views of the collar of the cotyledonary tube shown in Figs. 14, 16, respectively, note the papilla x 28: Fig. 20. A cotyledonary tube emerging from the collar, x 28. Fig. 21. Embryos at different stages of development during the seed germination, all x N. S. 3 note the well developed collar around the proximal haustorial part of the cotyledon and their distal part extending into the cotyledonary tube.

Col, Collar; *C.T.*, Cotyledonary tube; *pp*, papilla.

Ovary and ovule.—The ovary is superior. Generally there are two basal anatropous ovules (Fig. 18). Three ovules were also sometimes seen in *C. urens.* The tissue of the ovary is filled with raphide sacs, tannin and mucilage cells. There is a multicellular nectary in the basal region of the ovary (Fig. 11). The ovule is bitegmic and crassi-nucellate. The micropyle is mainly formed by the outer integument which is massive and is 6-10-layered. The inner integument is 2-3-layered. Multicellular glandular hairs are formed at the stigmatic surface at the time of fertilization (Fig. 22). A triangular epistase is seen during the post-fertilization stages and size the chalaza also shows overall increase in later.

Megasporogenesis.—This was studied by Rao (1959a) in this species. The archesporial cell is of the hypodermal origin. It divides to form 1-2 parietal layers, below which lies the functional megaspore (Fig. 20). It is broad at the micropylar end and tapering at the chalazal end. It divides [illegible] a linear tetrad (Fig. 19). [illegible] deposition of mucilage in [illegible] tetrad stage. The three [illegible] disintegrate and the [illegible] forms the embryo sac. [illegible]—The female game- [illegible] of the 8-nucleate [illegible] 21, 23, 24, 26). [illegible] and have lateral [illegible] synergids [illegible] Pl. [illegible] are [illegible] many. [illegible] same [illegible] during [illegible] Fig. 26). [illegible] the antipodal [illegible] deposition of tannin [illegible] embryo sac is about 90-102 x 50-170 μ.

Fertilization.—This is porogamous. The transmitting tissue is well developed in all the species (Fig. 18). One of the two synergids is crushed during the process of fertilization. A number of unfertilized eggs were noticed in several flowers on an inflorescence, but there was no seed formation.

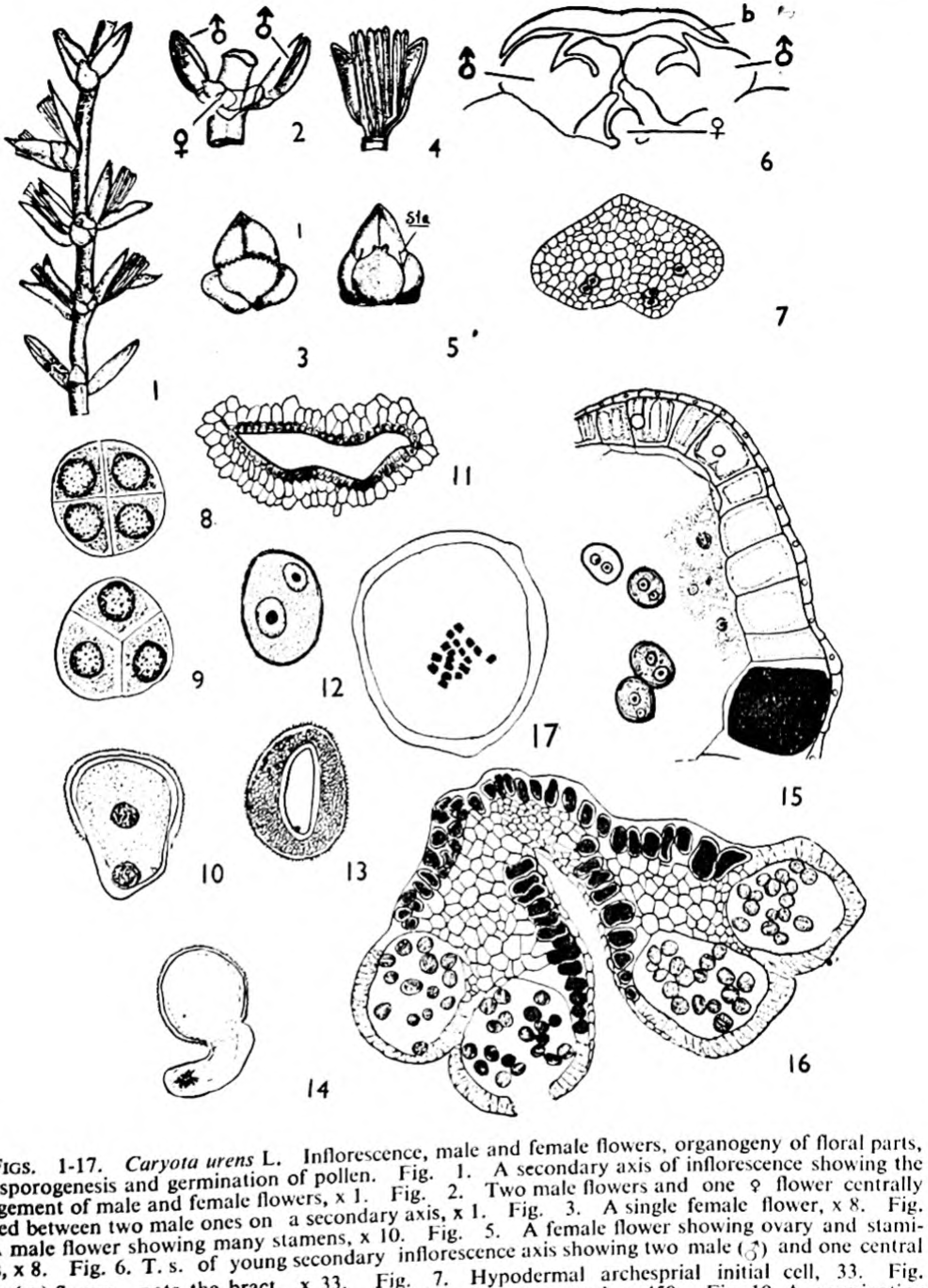
Embryo development.—The resting stage of the egg is considerably long. The first division of the zygote is transverse forming the basal cell *cb* and the terminal cell *ca* divided by an oblique wall (Pl. I, Figs. 6, 8; Figs. 33, 35).

The components of *ca* divide by transverse walls and form two tiers of cells-*q*-which in future give rise to the suctorial or haustorial part of the cotyledon by the time the seed is shed.

The basal cell *cb* divides by a vertical wall forming two cells (Fig. 34). These two cells give rise to *ci* and *m* by transverse divisions. The middle cell *m* appears to give rise to cells which form the future shoot (Figs. 41-43). Some cells of *m* also contribute to the formation of the proximal part of the mesocotyl. *ci* appears to give rise to a short suspensor, root initials and upper part of the mesocotyl region (Pl. II, Figs. 10-13; Figs. 36-40).

Major part of the development of the root and shoot takes place after the seeds have been shed. The growth and vasculature of the seedling during germination, therefore, undergo important changes in a growing embryo (Pl. II, Fig. 21; Figs. 44-52). The seeds take 3-4 months to germinate in moist mulch. Under natural conditions they mostly germinate in rainy season. Their mode of germination in *Caryota* is *Remotive.*

Endosperm.—The endosperm in *Caryota* is ruminated (Periasamy, 1959) and is of



FIGS. 1-17. *Caryota urens* L. Inflorescence, male and female flowers, organogeny of floral parts, microsporogenesis and germination of pollen. Fig. 1. A secondary axis of inflorescence showing the arrangement of male and female flowers, x 1. Fig. 2. Two male flowers and one ♀ flower centrally situated between two male ones on a secondary axis, x 1. Fig. 3. A single female flower, x 8. Fig. 4. A male flower showing many stamens, x 10. Fig. 5. A female flower showing ovary and staminodes, x 8. Fig. 6. T. s. of young secondary inflorescence axis showing two male (♂) and one central female (♀) flower, note the bract, x 33. Fig. 7. Hypodermal archesprial initial cell, 33. Fig. 8. A tetrahedral tetrad, x 450. Fig. 9. An isobilateral spore tetrad, x 450. Fig. 10. A germinating pollen grain, x 450. Fig. 11. T. s. of a floral nectary at the base of style, x 66. Fig. 12. A pollen grain showing generative and vegetative nuclei, note the reticulate exine, x 450: Fig. 13. A monocolpate pollen grain, x 450. Fig. 14. A pollen tube showing dividing generative nucleus, x 450. Fig. 15. A portion of the microsporangium showing 2-nucleate pollen grains. The endothecium, the dissolving tapetal cells and a large cell of endothecium containing tannin, x 200. Fig. 16. T. s. of anther showing dissolution of cells separating two microsporangia on each side, note the rows of tannin containing cells of the endothecium and also a number of pollen grains and the dehiscing wall of the anther lobe, x 180. Fig. 17. First metaphase showing 18 bivalent chromosomes, x 250.

b, bract; sta, staminode.

the *Myristica* type. Voigt (1888) found it to be of the *Myristica type* in *C. furcata.*

The ruminations are formed by the excessive growth of the outer-most layer of the *inner integument* (Voigt, 1888). Endosperm development studied here in *C. urens* conforms to the *Myristica* type.

Endosperm to begin with at the 4-celled stage of the embryo is free-nuclear. The endosperm nuclei seem to aggregate more at the antipodal part of the embryosac. At the octant stage the nuclei increased enormously and get arranged at the periphery of the embryosac (Pl. I, Fig. 4) as also at the antipodal end. The endosperm becomes cellular later by the process of vacuolization. Vacuoles appear around the endosperm nuclei. They press against each other and the cytoplasm between them thickens with middle lamellae. It is wavy to start with. Futher divisions in the endosperm cells are followed by cell plate formation (Rao Venkata, C. 1959a). A considerable variation was seen in the size of the endosperm nuclei (Figs. 27-30).

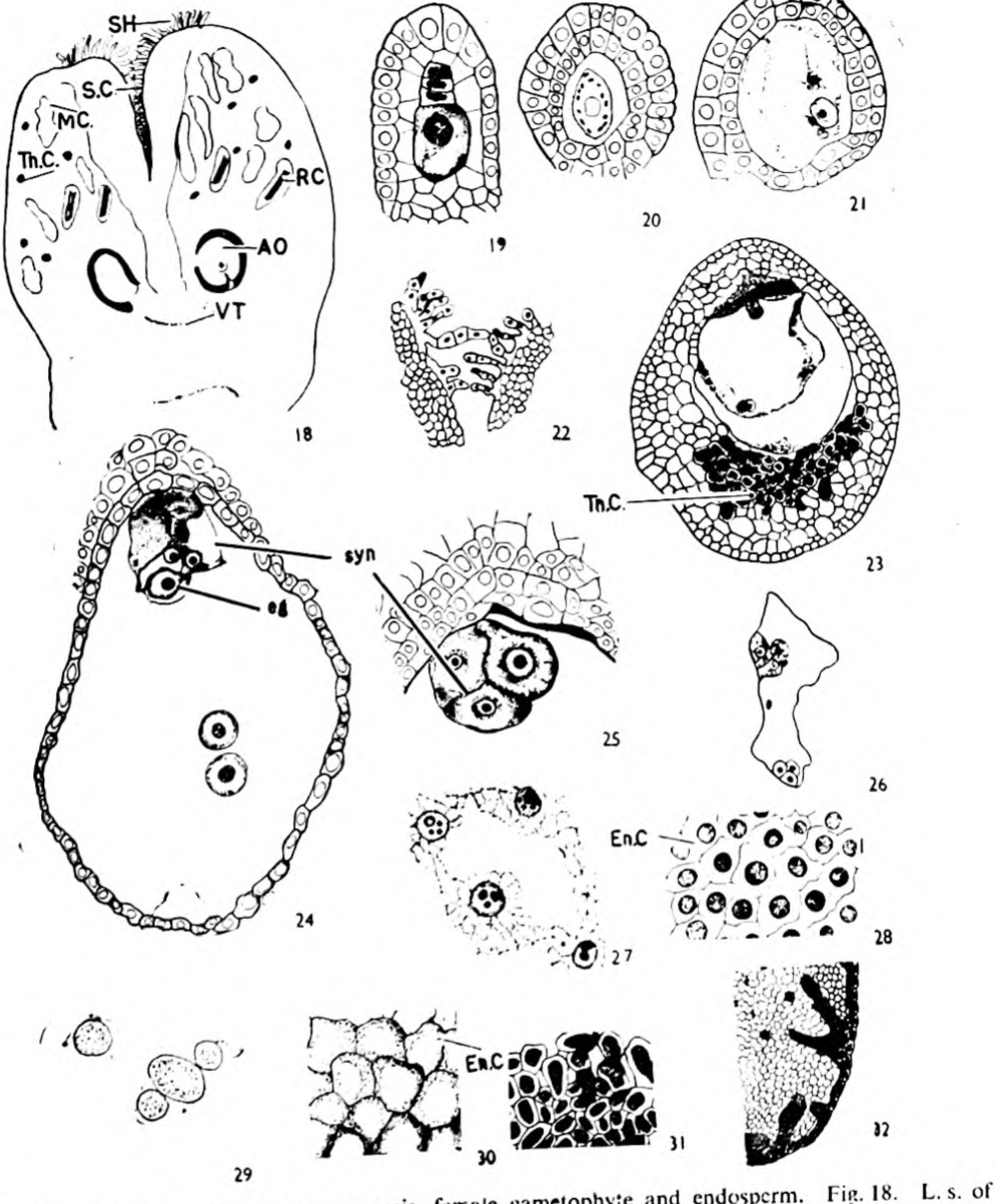
At the octant stage, the inner integument sends out protuberances which get filled with tannin. They were observed to push in at places inside the embryo sac. The inner layers of the inner integuments were not seen to take part in the formation of ruminations. The formation of ruminations from outer layers of the inner integuments was observed by earlier workers also (Voigt, 1888) and seems to be correct also from our observations (Figs. 31, 32).

At the globular stage of the embryo the seed grows considerably in size, though the embryo is very insignificant. The ruminations are conspicuous and they lie free in the entire peripheral part of it, extending at places upto the central part.

At the time of shedding of fruits, the ruminations become dark black due to deposition of tannin. The seed coat becomes very tough at this stage. The embryo is lodged at its periphery encircled by ruminations. During germination the proximal part of the suctorial cotyledon remains inside the seed and absorbs nourishment by developing finger-like haustoria (Figs. 53 and 54). They penetrate between the ruminations and absorb nourishment from the hemicullar layers, possibly by secreting some enzymes.

Seed germination.—A major part of the development of embryo takes place only after the seeds have been shed. Seeds are dark brown in colour and germinate readily in suitable places in rainy season e. g., in rotten leaves and mulch above the ground in forests, and about a foot or so below the ground near a parent tree from which the seeds have fallen. All the stages of germination are gone through in about 3-4 months and a seedling with first photosynthetic leaf, embryonic shoot and adventitious roots appears by that time.

The epicarp decays during germination and mesocarp absorbs water and becomes very loose and slimy. The micropylar region is slightly lifted up. Previously it used to look like a depression on the seed (Pl. II, Fig. 14). Lifting up of the micropylar region is a sure indication of germination. Next visible step in germinations is the emergence of the distal part of the cotyledon. A small circular disc or a collar is formed outside the seed simultaneously (Pl. II, Figs. 14-19). It is spongy and gets much frilled by absorbing moisture (Pl. II, Figs. 18, 19; Text Figs. 65-72). Next, as noted earlier, the root and shoot initials, lodged in the depression at micropyle slowly protrude out (Pl. II, Fig. 20). At this stage they are in flush with the



FIGS. 18-32. Ovary, megasporogenesis, female gametophyte and endosperm. Fig. 18. L. s. of ovary showing two basal ovules, x 525. Fig. 19. L. s. of ovule showing a megaspore, tetrad, x 450. Fig. 20. L. s. of ovule showing a megaspore, x 200. Fig. 21. L. s. of ovule showing uni-nucleate embryo sac, x 200. Fig. 22. Stigmatic hairs, x 450. Fig. 23. L. s. of ovule showing four-nucleate embryo sac, x 200; note the tannin cells. Fig. 24. L. s. of ovule showing eight-nucleate embryo sac showing egg apparatus with egg, and hooked synergids, x 300. Fig. 25. Egg-like synergids, x 100. Fig. 26. L. s. of ovule showing 8-nucleate embryo sac, x 300, note the shape of the embryo sac and the position of polar nuclei. Fig. 27. Endosperm nuclei developing protoplasmic strands around them, x 250. Fig. 28. Cellular endosperm, x 250. Fig. 29. Free-nuclear endosperm, x 250, note the size of the nuclei Fig. 30. Endosperm cells with nuclei disappearing, x 250. Fig. 31. Endosperm in a mature seed, note that the cells here are more or less completely filled with tannin, x 250. Fig. 32. L. s. of seed (a portion only) showing ruminations in endosperm, x 33.

Ao, anatropous ovules; *En. C*, cellular endosperm; *Eg*, *Egg*; *Mc*, mucilage canal; *Rc*, raphide sac; *Sc*, Stylar canal; *SH*, Stigmatic hair; *Syn.*, hooked Synergids; *Tn. C*, tannin cells; *VC*, Vascular trace.

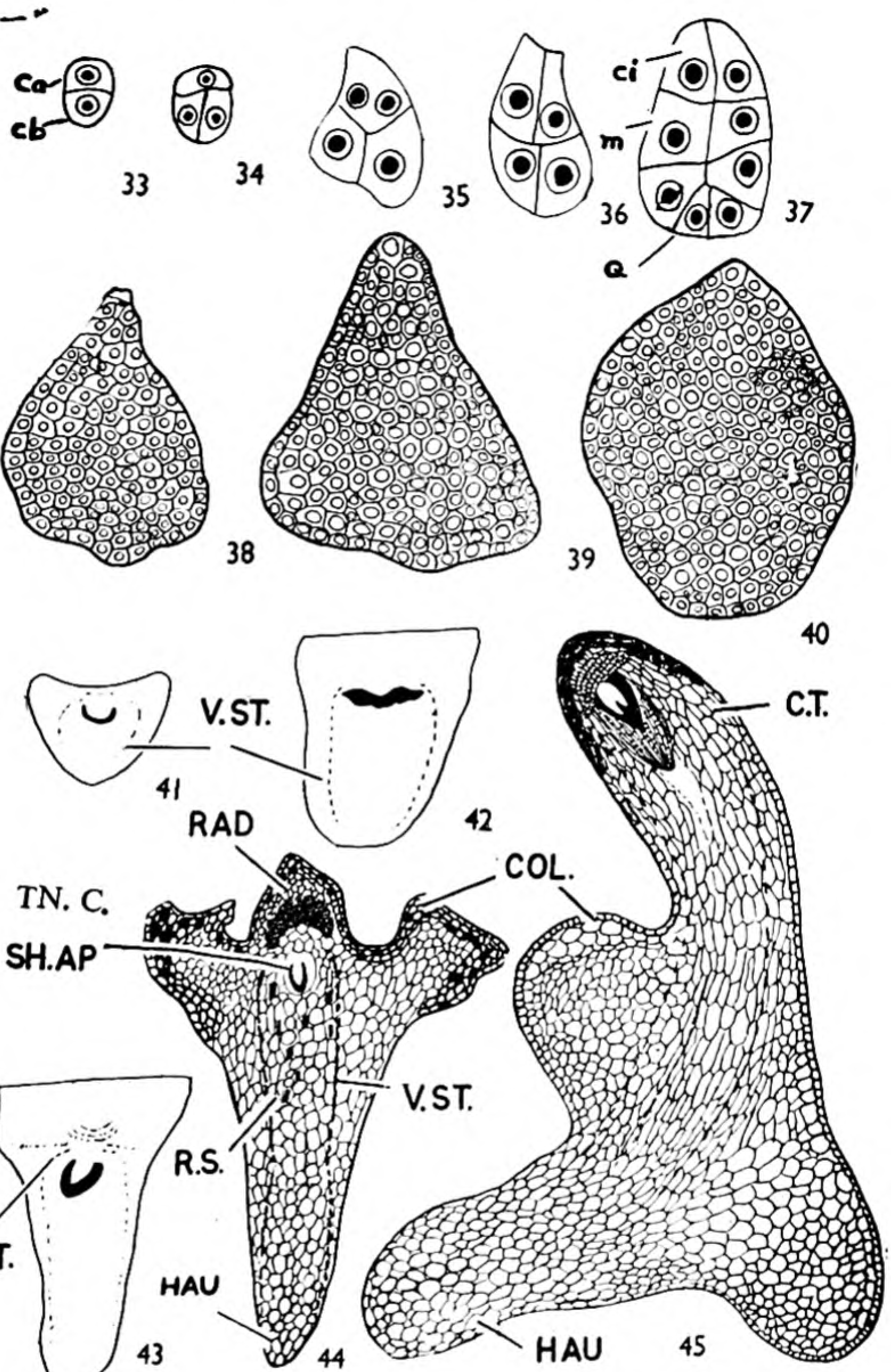
growing collar. The collar is the peripheral expanse or proliferation of the base of the distal part of the cotyledon outside the endocarp (Figs. 43-45). It serves as a canopy over the growing protrusion of the shoot and root node. From the nature of its cells, this tissue seems to be the rootcap region or the coleorhiza protecting the growing apex of the primary root (Figs. 44, 45). As the collar grows sideways its edges become more and more frilled. The collar thus seems to be a modified portion of the circular edge at the base of the haustorial portion of the cotyledon inside the seed and lies above the cotyledonary tube (Figs. 46-53 and Pl. II, Figs. 14-21). A root papilla lies at its centre (Figs. 46, 47, Pl. II, Figs. 15, 16, 18, 19).

Next, a tube-like structure is formed which carries the root and shoot initials in it. The distal end of this tube lies inside the seed and its basal portion or the end grows outside the seed. The meristem around the embryonic shoot and root axis is a well-knit closed unit, and it comes out of the seed together. It continues to function as a unit for quite some time. In early stages of development, root pole is differentiated less, but the shoot pole grows precociously (Pl. I, Fig. 7). The tube-like structure, therefore, is only a continuation of the central part of the cotyledon which has undergone maximum elongation (Pl. II, Fig. 21, Figs. 46-52). A cavity is formed at its centre lysigenously and is seen above this unit, inside the cotyledonary tube (Fig. 72-73). The collar lies at its other end near the eye on endocarp externally.

The formation of cotyledonary tube starts with the formation of an oblique slit. Three morphologically distinct regions of the cotyledon get differentiated (Figs. 53 a, b, c, d —57): (a) the proximal part forming the future suctorial or haustorial organ inside the seed, (b) the distal part forming a circular elongated spongy region outside the eye on pericarp as an epinodal tube-like elongation and (c) the collar which is a continuation of the first or embryonal nodal region and its periphery. The root-shoot unit is carried in the cotyledonary tube below the collar (Figs. 43-44, 53, 58, Pl. I, Fig. 9). The slit lies next to the shoot initials. The cotyledonary tube emerges out of the seed along with the sheathing part of the collar at the distal end which is the root region. Its proximal part continues to grow slowly and a special kind of meristomatic ring is formed around the first node and above the collar which initiates the growth and elongation of the cotyledonary tube.

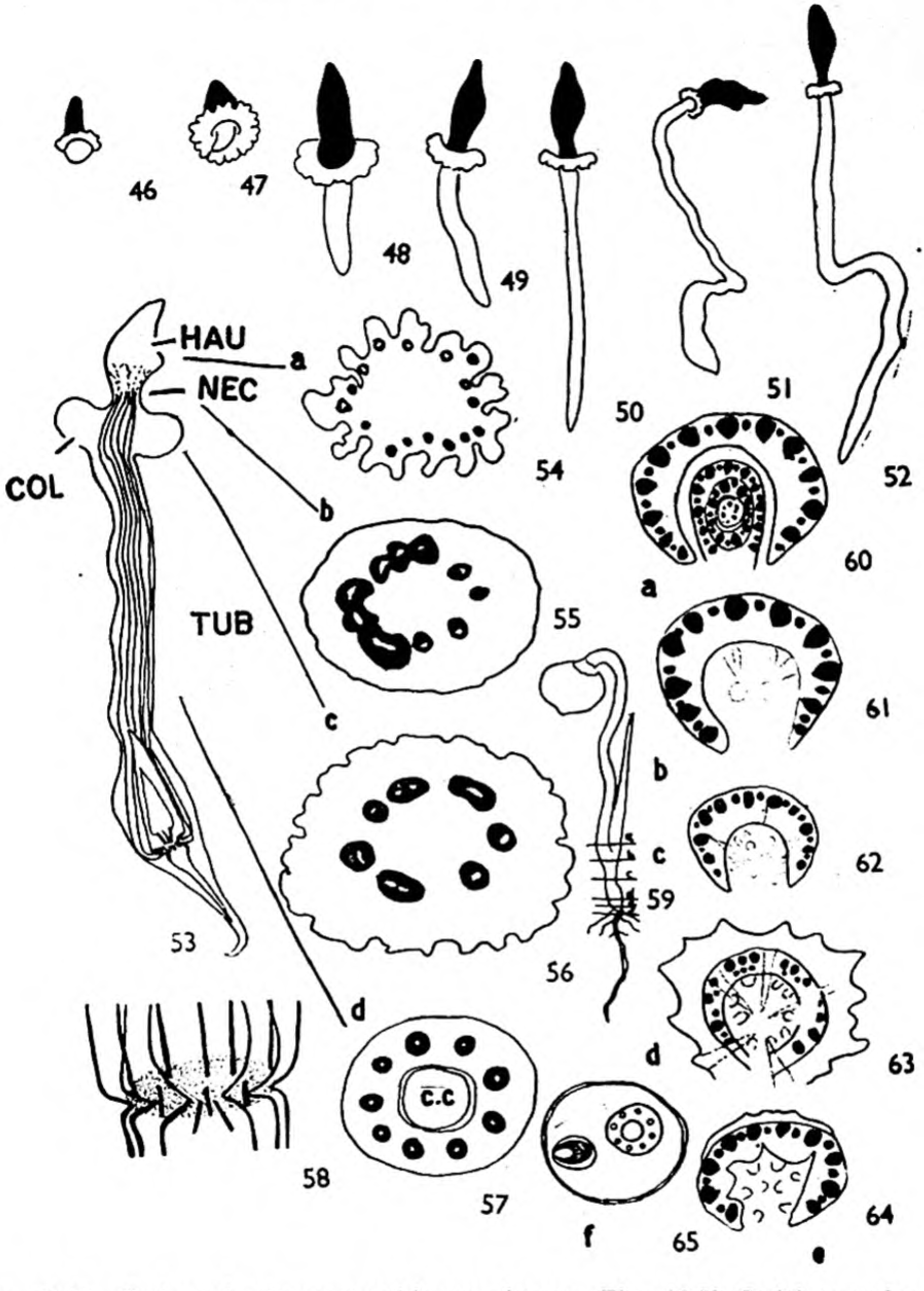
At the extreme tip of the tube outside the seed lies the coleorhiza. Just above this lies the short shoot apex, and next to it and around and above it lies the gutter-like portion of the middle piece of the single cotyledon. It transmits dissolved food material absorbed by the sucker cells of the haustorial part of the cotyledon inside the seed at the proximal end, as well as by the cells of the collar outside the seed lying embedded in the moist mulch on the ground in which the seed germinates. The cotyledonary tube, therefore, can safely be considered as the hypocotyl or the lower piece of the functional single cotyledon and the collar its middle piece or the mesocotyl (Fig. 53, Pl. II, Fig. 21).

Vasculature of the seedling.—The first hypocotyledonary node in the embryo is differentiated at a very early stage when the seed is about to be shed. A number of procambial strands run from this nodal ring to the proximal part of the cotyledon and go to epiphysis. The vascular supply going to the collar is very poor or nil.



FIGS. 33-45. Embryo development. Fig. 33. Two-celled embryo, x 400: Fig. 34. Three-celled embryo, x 200. Fig. 35. Four-celled embryo, x 200. Fig.36. Further divisions in an embryo, note *cb* forming *ci* and *m* cells. *ca* gives rise to quadrant-*q*, x 200. Figs. 38, 39. Globular stages of embryo formed by anticlinal and periclinal divisions of embryonic cells, x 185. Fig. 40. A further stage of growth of embryo, x 200. Figs. 41, 42. L. s. of an embryo showing plumule and vascular traces, x 400. Fig. 43. L. s. of an embryo in a seed during early germination, x 200. Fig. 44. L. s. of an embryo during further germination of seed, note the expanded collar, radicle lodged in the cotyledonary tube, the developing shoot apex, haustorial part of the cotyledon, the raphide sac and the tannin containing cells x 200. Fig. 45. A further advanced stage of embryo. Note the collar. Cotyledonary tube (with root and shoot in it) and the haustorial part of the cotyledon, x 200:

COL, collar; *CT*, cotyledonary tube; *HAU*, haustorial part of the cotyledon; *RAD*, radicle; *R.S.*, raphide sac; *SH. AP.*, developing shoot apex; *TN. C.*, tannin containing cells; *V. St.*, vascular traces.



FIGS. 46-64. Embryo development and its vasculature. Figs. 46-52. Serial stages in a developing embryo during germination of seed. Note the development of the cotyledonary tube at the distal end and its haustorial part (made dark) at the proximal end. In between them lies the collar, x 2. Fig. 53. L. s. of an entire seedling, note the haustorium the neck-like portion of the cotyledonary tube, the collar and the cotyledonary tube with vascular traces, x 3. Fig. 54. T. s. of the haustorial part showing wavy margin and poorly developed vascular tissue, x 18. Fig. 55, 56. T. s. of collar showing frilled margin and seven fibrovascular bundles, x 18. Fig. 57. T. s. of the cotyledonary tube showing central cavity and 9 vascular strands, x 18. Fig. 58. L. s. of the nodal plate showing vasculature of the shoot and root, x 60. Fig. 59. A germinating seedling showing emergence of non-photosynthetic leaf and roots, x 2. Fig. 60-64. T. s. of a seedling in Fig. 59. at the levels, a, b, c, d, e and f, note the vascular supply to cotyledon at the levels in *a, b, c, d,* and *e, and* to root at the level *f,* x 60.

CC, central cavity; *COL,* collar; *HAU,* haustorium; *NEC,* neck like cotyledonary tube; *TUB,* vascular trace of cotyledonary tube.

The hypocotyledonary node in later stages after the root and shoot have been established shows swelling all round. A. L. S. at the nodal point as shown in Pl. I Fig. 9, Figs. 53, 58 shows 10-11 vascular strands going up, to the cotyledonary tube. The vascular strands in the neck region of the tube fuse in groups of 2, 3, or 4.

The root-stem region extends to about 5 mm in palm embryos as in other monocots. In *C. urens* the first photo-synthetic leaf appears after 3 months and has 3 major leaf traces as in *Yucca whipplei* (Arnott, 1962). T. S. of the petiole of the first photosynthetic leaf shows endarch xylem with collateral bundles.

The sequence of the development of xylem is, distal and middle parts of the tube first, and then in the root.

From the above anatomical and morphological observations it will be seen that the first leaf in *Caryota* is tubular and haustorial; the 2nd leaf is generally scaly and non-photosynthetic and the 3rd leaf is photosynthetic, and it has the characteristic fenestration (Figs. 59-65).

Embryo culture.—The embryo culture has become an important modern method for the rapid germination and growth of seeds, especially in orchids. The experiments given below were undertaken to see whether the embryo in *Caryota* could be isolated and grown satisfactorily in culture media, as the development of palm embryo under natural conditions takes considerable time often several months. But if they could be grown successfully in culture media, it would be of interest in commercial utilization of the palm seeds in nurseries and gardens. To the best of our knowledge very few attempts have been made in that direction in palms and hence this work was undertaken.

Mature embryos from seeds, 8-9 months old were dissected and taken out in a sterile culture chamber and implanted in culture bottles containing 35 ml of Knop's solution and other media. They were kept in an incubator at 28°C. pH of the media was adjusted to 5.5 previously and the response of embryos to various factors was noted. The other medium used was White's basal medium enriched with vitamines and $Fe_2(SO_4)_3$.

Two percent agar was poured hot in White's medium with adjustment of pH at 5·5. 3 ml solution of the above medium was poured in each bottle previously sterilized. Penicillin vials were used as culture bottles. They were plugged with cotton and wrapped in brown paper and autoclaved under 18 lbs pressure for 15 minutes. Culture vials were cooled and stored at 28°C. The dissected embryos were transferred to them and kept for growing. The growth noted was as shown in table I.

The results obtained from these were compared with the corresponding growth stages of embryos in seed sown in soil under natural conditions. It was seen that growth of embryos in them was much less. From this it may safely be said that the growth in culture media like White's standard basal medium or Knop's is not only satisfactory, but much more rapid than under the soil conditions. Hence this method needs to be tried on a large scale for the purposes of mass cultivation. However, this will need standardization of the technique of culturing embryos on a large scale. But the significant fact is that palm embryos when excised do grow well in culture media under laboratory conditions, and that this method is likely to prove useful for the quick propogation of the palms.

DISCUSSION

The mode of germination of seeds is of special significance in palms. It is of the *Remotive* type in *Caryota, Phoenix* etc. (Saakav, 1954). The young embryo in a ripe seed of *Caryota* is very inconspicuous; the major part of a seed is filled with ruminated endosperm. In palms with *Admotive* type of germination as in *Cocos* the primary root develops precociously compared to that in the *Remotive* type. As remarked by us earlier (Mahabale and Shirke, 1968), the Caryotoideae form an intermediate group between the pinnate and palmate-leaved palms, or between the reduplicate and induplicate-leaved palms. The embryo development in this genus is of the *Asterad* type as in *Phoenix*. In later stages after the globular stage, embryo has two lobes of cotyledons, which fuse in the distal part of the cotyledon. It grows out into a frilled collar outside the endocarp and the root-shoot primordia come out with it and turn at an angle of 45° and more towards the soil. A tube-like growth comes out from the distal end of the cotyledon and continues to grow until the primary root and shoot are established. The shoot emerges obliquely splitting the cotyledonary tube. In the embryology of several palms a similar tube-like cotyledonary organ is formed. It is essentially a sheathing leaf. Ontogenetic studies on it show that it has tomentum on it. It's extreme distal part splits open and the stem or shoot apex emerges from it. It has vasculature typical of a cylindrical leaf. Morphologically, therefore, the cotyledonary tube is an outgrowth from upper part of the first root-stem node forming a closed sheathing leaf. The nodal plate is 0.5 mm in length. The vascular traces of the cotyledonary tube and those of the root are continuous and they emerge in opposite directions from the primordial nodal plate (Pl. I, Fig. 9, Fig. 58).

TABLE I

(1) *Medium used* ; KNOP'S SOLUTION

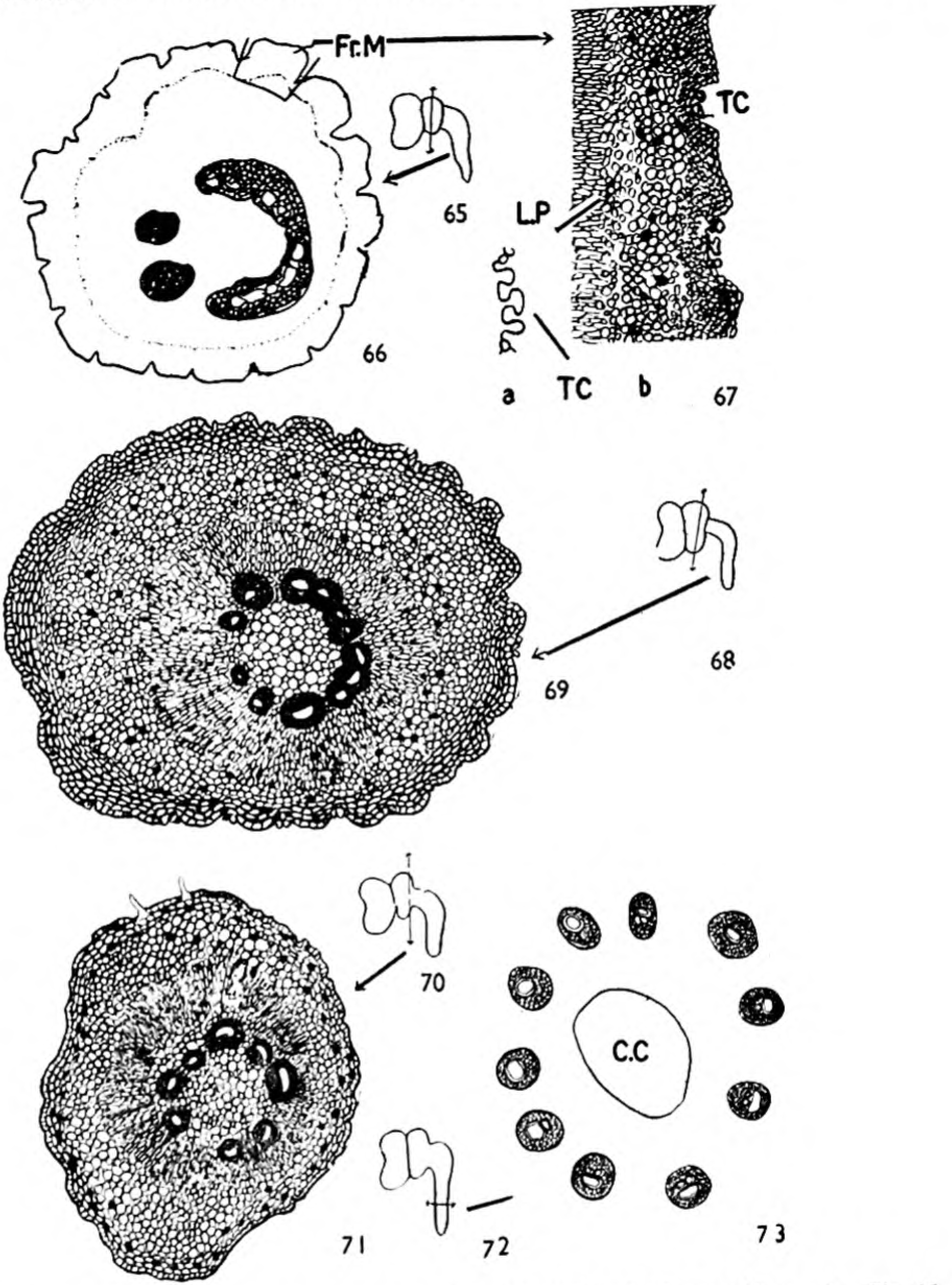
Date	Initial length	Duration	Growth in mms
3-2-61	4×3		
3-3-61	6×3	1 month	2×1 mm
3-4-61	8×5	2 months	4×3 mms

Growth in 1 month=2×1 mm
Growth in 2 months=4×3 mm

(2) *Medium used* : WHITE'S BASAL MEDIUM PLUS 2% AGAR

Date	Initial length	Duration	Growth in mms
3-2-61	4×2	1 month	
3-3-61	6×4		2×2 mms
3-4-61	8×4	2 months	4×4 mms

Embryologically, *Caryota*, therefore, seems somewhat closer to *Phoenix* than to



FIGS. 65-73. Structure of the cotyledonary collar. Fig. 65. A germinating seedling, x ½. Fig. 66. T. s. of collar at the level shown in Fig. 65, showing the frilled margin and central fused fibrovascular bundles, x 62. Fig. 67. T. s. of a portion from the peripheral part of the collar, x 100: the turgid cells and the loose parenchymatous cells, x 100. Fig. 68. A germinating seedling, region of the collar through which this section was taken is indicated by the arrow shown in Fig. 69. x ½. Fig. 69. T. s. taken at the region shown by the arrow in Fig. 68, x 62. Fig. 70. A germinating seedling, Position of the arrow here shows the level at which the section of the collar was taken, x 1. Fig. 71. T. s. of the collar at the level shown by the arrow in Fig. 70, note the epidermal hairs and the central vascular tissue, x 62, Fig. 72. A germinating seedling, note the region of the cotyledonary tube indicated by the arrow, x ½. Fig. 73. T. s. of the cotyledonary tube at the level shown by arrow in the Fig, 72, note the ten vascular strands with well developed proto-and meta-xylem elements, and the central cavity, x 62.

C.C., central cavity; *Fr. M*, frilled margin; *LP*, loose parenchymatous cells; *TC*, turgid cells.

the palmate palms like *Licuala*, or *Livistona*. *Phoenix* and *Caryota* both have *Polygonum* type of 8-nucleate embryo sac, *Asterad* type of embryo development e. g. in *Phonix silvestris* Roxb., *P. pusilla* Gaertn., *P. acaulis* Buch., and *P. reclinata* Jacq., and *Remotive* type of seed germination as that in *Phoenix*, besides other points of similarity in their morphology such as induplicate vernation of leaf etc., as indicated in the first part of this study (Mahabale and Shirke, 1968, p. 484). *Caryota*, however, differs from it radically in having inflorescences which are hermaphrodite. They open basipetally on a tree, which is a unique character of the genus *Caryota*. The endosperm in this genus also is characteristically ruminate but not so in *Phoenix*. The fruit in *C. urens* generally has two seeds but one in *Phoenix*. The affinity between these two genera, however, must be rather remote. The collar formed during seed germination in this genus is also another unique feature of *Caryota* as was remarked by one of us. (Mahabale, 1966 p. 172-175).

REFERENCES

ARNOTT, H. J. 1962. The Seed, germination and seedling of *Yucca*. *Univ. Calif. Publs. Bot.* **35**: 1-164.

BIRADAR, N. B. 1968. Embryology of *Phoenix pusilla* Gaertn., *P. acaulis* Buch. and *P. reclinata* Jacq. *Proc. Indian Acad. Sci.* B **67**: 165-173.

DE POERCK, R. A. 1950. Contribution a l' étudé de palmier a huile African (*Elacis guineensis* Jacq.) *Ole. aegineux* **5**: 623-28.

JULIANO, J. B., AND E. QUISUMBING. 1931. Morphology of the male flower of *Cocos nucifera* L. *Philipp. J. Sci.* **45**: 449-58.

KAJALE, L. B., AND S. G. RANADE. 1952. Occurrence of four kinds of tetrads in *Elaeis guineensis* Jacq. *Curr. Sci.* **27**: 170.

——,—,AND—. 1953. The embryo sac of *Elaeis guineensis* Jacq. An investigation. *J. Indian bot. Soc.* **32**: 101-107.

KULKARNI, A. R. 1965. *Studies on the Genus Cocos.* thesis Poona University,

MAHABALE, T. S. 1966. The embryo in Palmae. In *Proceedings of the Autumn School in Botany*, University Grants Commission, New Delhi and the University of Poona **163**-185.

MAHABALE, T. S., AND M. S. CHENNAVEERAIAH. 1957. Studies on *Hyphaene indica* Becc. I. Morphology. *Phytomorphology* **7**: 184-194.

—, AND N. B. BIRADAR. 1968. Studies on palms: Embryology of *Phoenix sylvestris* Roxb. *Proc. Indian Acad. Sci.* **B 67**: 77-96.

—, AND NANDINI SHIRKE 1968. The genus *Caryota* in India. *J. Bombay nat. Hist. Soc.* **64**: 462-487.

PERIASAMY, K. 1959. The ruminate endosperm development and types of rumination. In *Plant Embryology. A. symposium.* C.S.I.R., New Delhi.

QUISUMBING, E., AND J. B. JULIANO. 1927. Development of ovule and embryo sac of *Cocos nucifera* L. *Bot. Gaz.* **84**: 279-93.

RAO, VENKATA, C. 1955. Embryo development in *Areca catechu*. *Nature, Lond.* **175**: 432.

——. 1959a. Contributions to the embryology of Palmae. Part., I, Sabaleae. *Proc. natn. Inst. Sci. India* **29**: 134-164.

——. 1959b. Contributions to the embryology of palmae. Part II. Ceroxylineae, *J. Indian bot. Soc.* **38**: 46-75.

SAAKAV, S. R. 1954. *The Palms and Their Culture in U. S. S. R.* Academy of Sciences, U.S.S.R., Leningrad, Moscow (in Russian).

SCHNARF, K., 1931. *Vergleichende embryologie der Agiospermen.* Berlin.

SELVARATHNAM, G. M., 1952. Embryo of coconut. *Nature, Lond.* **169**: 714.

SHIRKE, NANDINI S., 1966. Embryology in the genus *Caryota*. *Proc. 53rd Indian Sci. Congr. Chandigarh.* 262.

SWAMY, B. G. L. 1942. Embryological Studies in Palmae. A Preliminary note on the megasporogenesis in *Areca catechu* Linn. *Curr. Sci.* **2**: 199.

*VOIGT, A., 1888. Untersuchungen Uber Bau and Entwicklung von Samen mit ruminierten Endosperm aus den Familien der Palmen, Myristicaceen, und Annonaceen. *Ann. Jard bot. Buitenz.* **7**: 150-190.

---

*Not seen in the original.

Adv. Pl. Morph. 1972: 233-242.

PURIOXYLON JURASSICA GEN. ET SP. NOV. FROM AMARJOLA IN THE RAJMAHAL HILLS, INDIA.

By B. D. Sharma

Department of Botany, University of Jodhpur. India

ABSTRACT

Descritption is given of a newly discovered petrified gymnospermous wood, *Purioxylon jurassica* gen. et sp. nov. collected from the fossiliferous locality of Amarjola. Outer surface is longitudinally wrinkled and it is provided with sparsely placed and spirally arranged leaf bases. In the axil of each leaf base there is an axillary bud. In the peripheral portion of pith there is an internal periderm, surrounding which is present a vascular zone consisting of numerous, collateral, conjoint and endarch bundles. Secondary wood is manoxylic. Cortical bundles are concentric or collateral having protoxylem points exarch, endarch or mesarch. They all are provided with picnoxylic secondary xylem. Mucilage canals are present in the peripheral portion of cortex.

INTRODUCTION

The fossiliferous locality of Amarjola has yielded a large number of known plant groups like Cycads, Bennettitales, Conifers and Pentoxyleae (Sharma 1967, 1968, 1969b) besides, some of the interesting genera of doubtful affinities for example, *Sewardioxylon sahnii* Gupta (1971), *Guptioxylon amarjolense* Sharma (1969a) etc. The material of the present genus has also been collected from the same locality and for the present its affinities are also unknown, as it possesses some of the anatomical characters resembling Cycads while others are of the group Pentoxyleae. Relationship of the new genus with allied fossils and living genera is also discussed in the present paper.

The new genus has been named as *Purioxylon*, in honour of the great plant morphologist and an illustrious teacher, Prof. V. Puri of Meerut University, Meerut (India).

DESCRIPTION

Purioxylon GEN. NOV,

Diagnosis.—Stem is provided with sparsely placed and spirally arranged leaf bases, in the axil of each leaf base there is an axillary bud. In the peripheral portion of pith there is an internal periderm, surrounded by a vascular zone consisting of numerous collateral, conjoint and endarch bundles having manoxylic secondary xylem. Cortical bundles are concentric or collateral with exarch, endarch or mesarch protoxylem points. Cortical bundles are provided with picnoxylic secondary xylem. These bundles originate from the vascular zone and a number of cortical bundles are supplied to an axillary bud.

Purioxylon jurassica GEN. ET SP. NOV.

Diagnosis.—Outer surface of stem is longitudinally wrinkled. Leaf bases crescent shaped, 4 to 5 mm long; axillary bud circular, 3 to 5 mm in diameter. Internal periderm consists of rectangular cells. Vascular zone is 0.6—0·8 mm thick, made up of squarish tracheids arranged in rows. Secondary xylem is manoxylic. Cortical bundles are endarch in the beginning but later on they become exarch or mesarch. Cortical bundles are provided with picnoxylic secondary xylem without xylem parenchyma. In the peripheral portion of cortex there are mucilage canals.

Type specimen No. B.VI/Raj. A. Sharma Coll., Univ. Jodhpur.

Locality—Amarjola in the Rajmahal Hills.

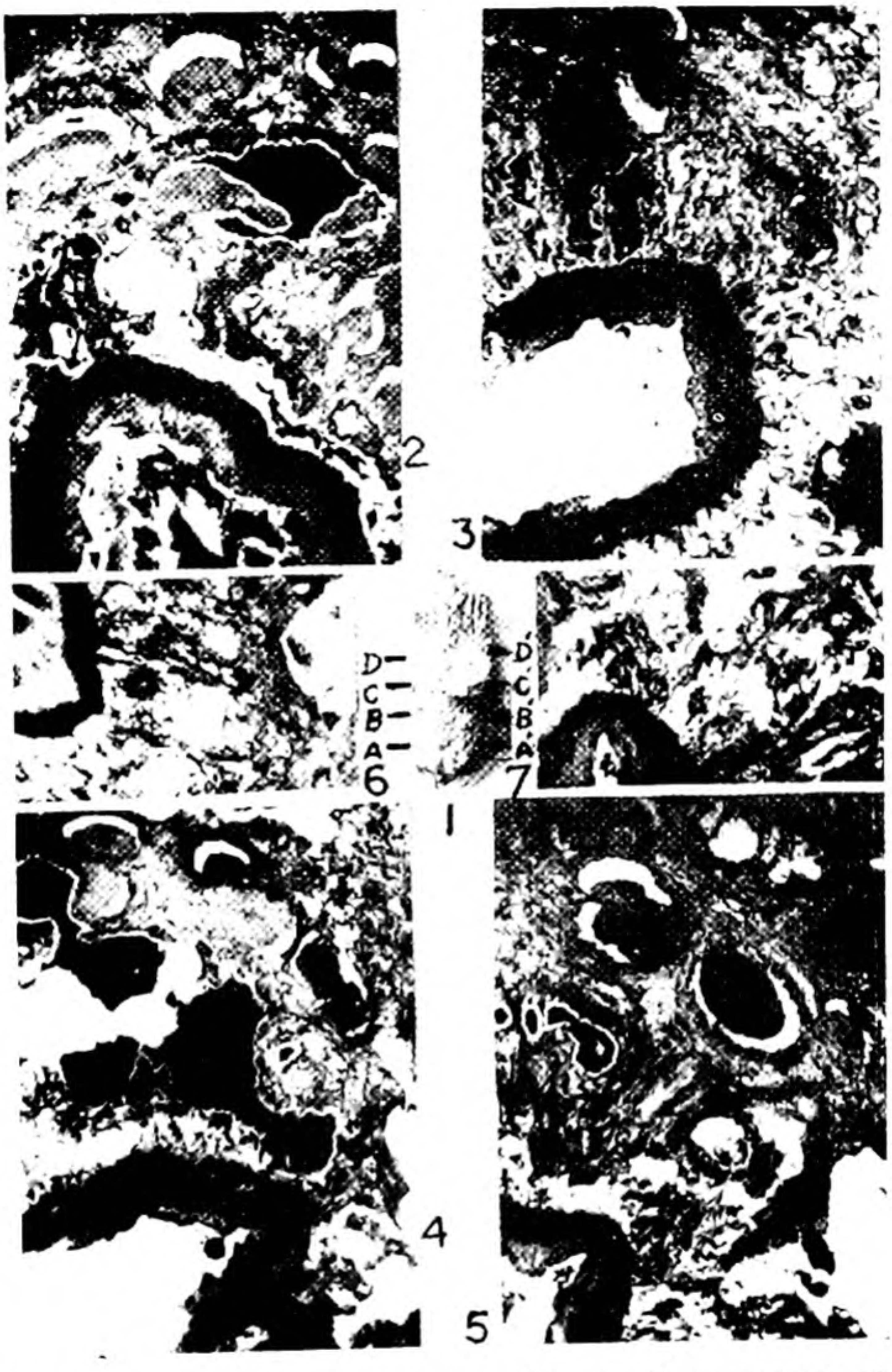
Horizon.—Jurassic.

Detailed description.—There are present a number of specimens of this species in author's collection; out of which the Type specimen No. B.VI/Raj. A. (Fig. 1) is the best preserved. It is a petrified piece of stem, 2.8 cm long and 1.2 to 1.7 cm in diameter. It is provided with raised, crescent shaped leaf bases; each is provided with an axillary bud.

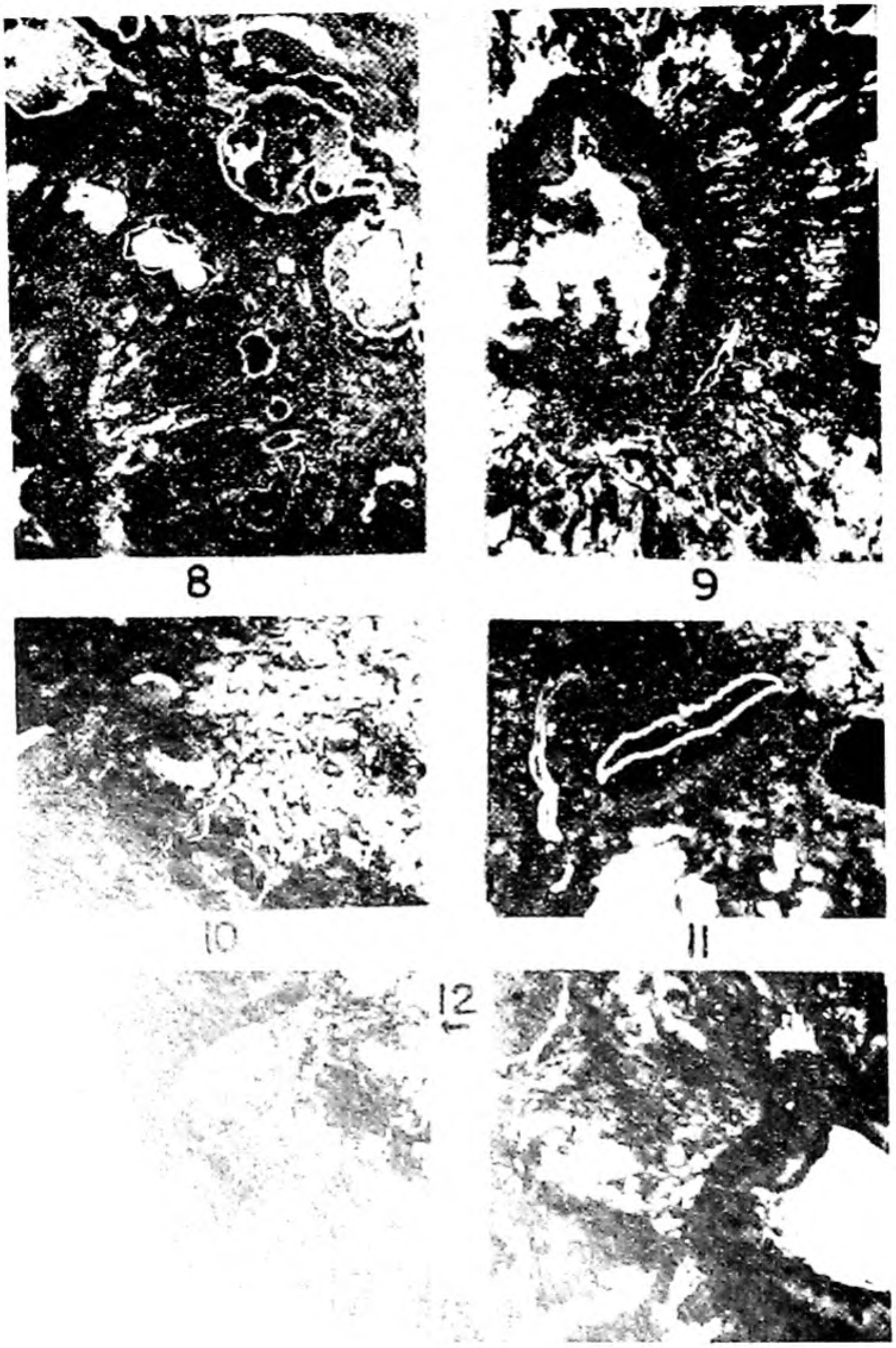
A series of transverse sections have been cut to study the changes taking place in the anatomy at the different levels in the stem.

A—A' (Fig. 21).—Transverse section at this level of the type specimen (Fig. 1) shows an unpreserved central portion representing the pith, surrounding which there is present a 300 to 400 μ thick, darkish layer of internal periderm (Figs. 3, 4, and 20). It is made up of rectangular cells which are arranged in rows. Surrounding the internal periderm there is a 600–700 μ wide, poorly preserved vascular zone (Fig. 3), which is made up of a large number of collateral, conjoint and endarch bundles (Fig. 21). Secondary xylem is manoxylic, consisting of squarish tracheids arranged in rows. In between the bundles there are 3–5 cells wide parenchymatous pith rays, Newly originating cortical bundles are seen lying opposite the medullary rays. In the beginning these bundles are more or less rounded having an endarch protoxylem point and are provided with only a little amount of picnoxylic, secondary xylem (Fig. 10). During their way through the cortex, these bundles are not only gaining the secondary xylem but also changing in their shapes and sizes ranging from 80 x 140 to 450 x 600 μ (Figs. 10-12, 15-19). Cortical bundles are either concentric (Fig. 4) or collateral (Fig. 11) having protoxylem points exarch (Fig. 16), endarch (Fig. 11) or mesarch (Fig. 4). Some of the cortical bundles are fusing with each other while others are seen dividing and forming new cortical bundles (Fig. 19). All the cortical bundles are provided with picnoxylic secondary xylem like the one found in *Pentoxylon* Srivastava (1946), but unlike the latter, growth rings are not differentiated. In the peripheral portion of cortex there are present a number of mucilage canals (Figs. 15, 16, 18), each consisting of 3-5 cells.

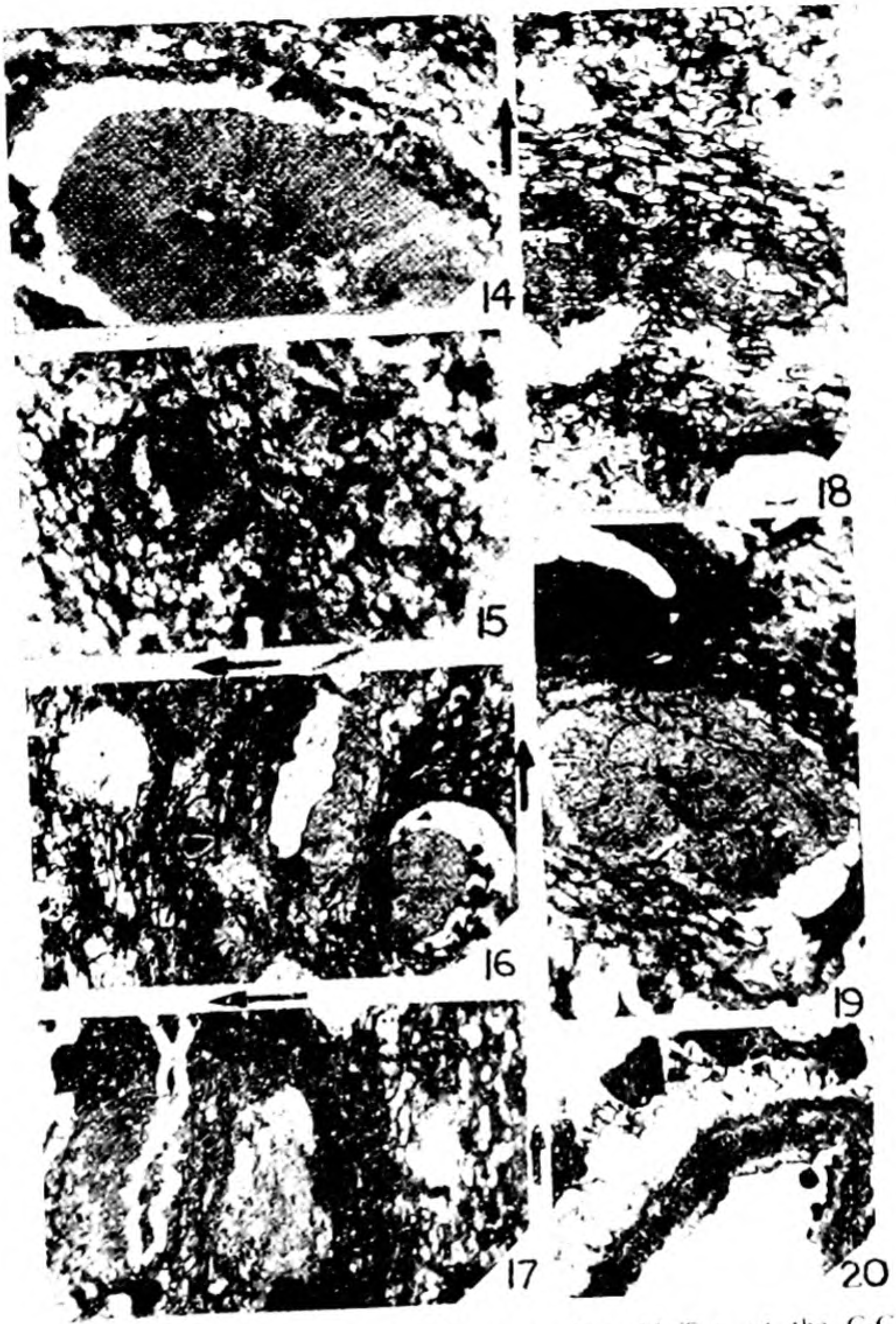
B—B' (Fig. 22).—The transverse section at this level of the stem shows the anatomy more or less, the same as seen in the previous slide, except that some changes have taken place in the number arrangement, shape and size of the cortical bundles. These bundles are placed irregularly and in more than one row (Fig. 2). One of the concentric type of cortical bundle is comparatively very



FIGS. 1-7. *Purioxylon jurassica* gen. et sp. nov. Fig. 1. Type specimen No. B. VI/Raj. A. showing external morphology and different levels of sectioning (A-A′ to D-D′), x 3/4 Fig. 2. T. s. at the B-B′ level showing a darkish layer of internal periderm and a number of collateral and endarch cortical bundles, x 18. Fig. 3. T. s. at the level A-A′, showing a poorly preserved vascular zone outside the internal periderm and the originating cortical bundles, x 18. Fig. 4. T. s. same, showing cortex of the other side having cortical bundles of different sizes, x 18. Fig. 5. T. s. at the D-D′ level showing two concentric bundles in the cortex, one of them is seen dividing, x 18. Fig. 6. T. s. same, showing collateral bundles of vascular zone with unpreserved phloems. Bundles are separated by rays, x 18. Fig. 7. T. s. same, showing a portion of the vascular zone having manoxylic wood, x 18.



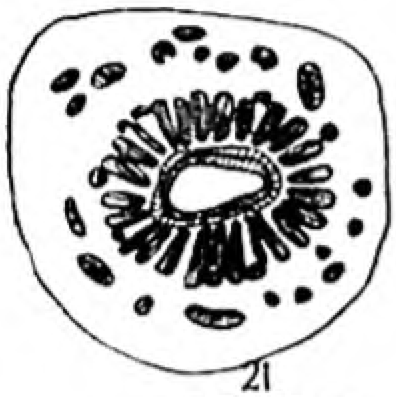
[illegible] T. s. at the D-D′ level showing collateral [illegible] areas on the outerside representing phloem, × 18. Fig. [illegible] periderm and the surrounding manoxylic vascular zone, × [illegible] showing cortical bundles of different shapes and sizes, × 18. [illegible] vascular zone outside the internal periderm and a concentric cortical [illegible]

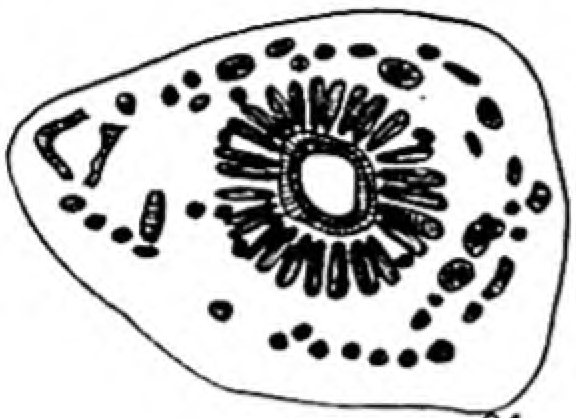


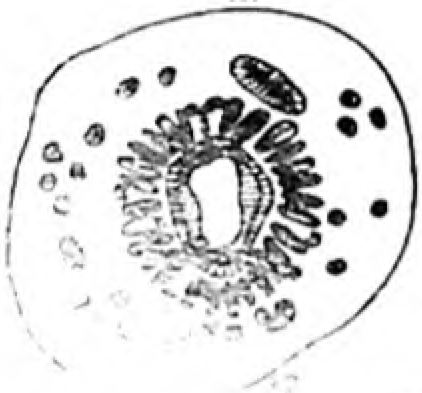
FIGS. 14-20. *Purioxylon jurassica* gen. et sp. nov. Fig. 14. T. s. at the C-C′ level showing a concentric cortical bundle with picnoxylic secondary xylem, x 54. Fig. 15. T. s. at the A-A′ level showing a portion of the cortex having a small sized cortical bundle, x 54. Fig. 16. T. s. same, showing a portion of cortex having exarch (inner rounded one) and endarch (outer one) bundles, x 54. Fig. 17. T. s. same, showing a portion of cortex having two bundles with different orientations, x 54. Fig. 18. T. s. same, showing outer portion of cortex with two mucilage canals and small sized cortical bundles, x 54. Fig. 19. T. s. same, showing a dividing cortical bundle, x 54. Fig. 20. T. s. same, showing a portion of internal periderm surrounding an unpreserved cavity representing the pith, x 18.

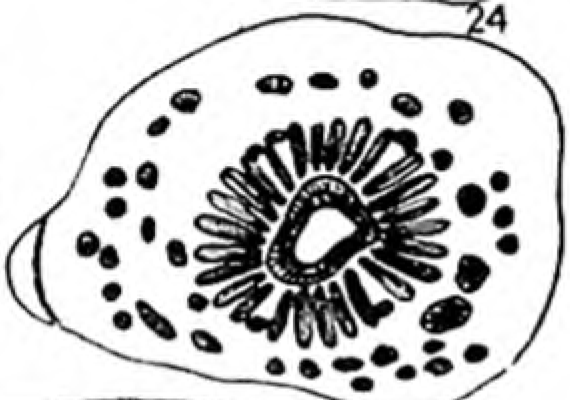
large, 500×700 µ in size (Fig. 22). It is provided with 230 µ thick layer of picnoxylic secondary xylem all around the primary xylem. Primary xylem is 2-3 cells thick and consists of more or less, circular cells.

C—C′ (Fig. 23).—This slide shows the transverse section passing through the leaf base. Unfortunately, the cellular structures in the leaf base are not clear, Otherwise, the general anatomy of the stem remains the same as given above. A large number of cortical bundles of different sizes are present in the portion of the cortex lying adjacent to the leaf base (Fig. 27). Newly originating cortical


21


24

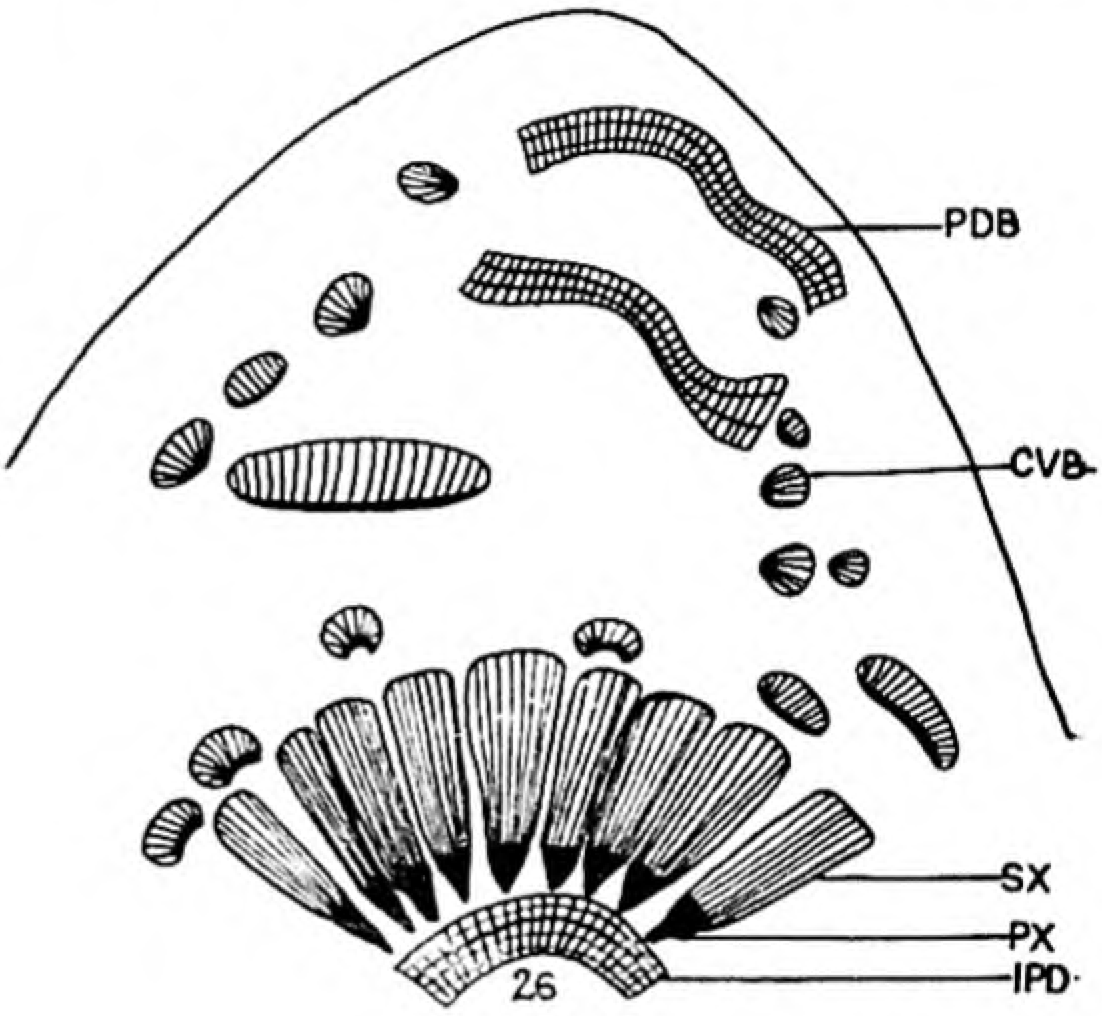


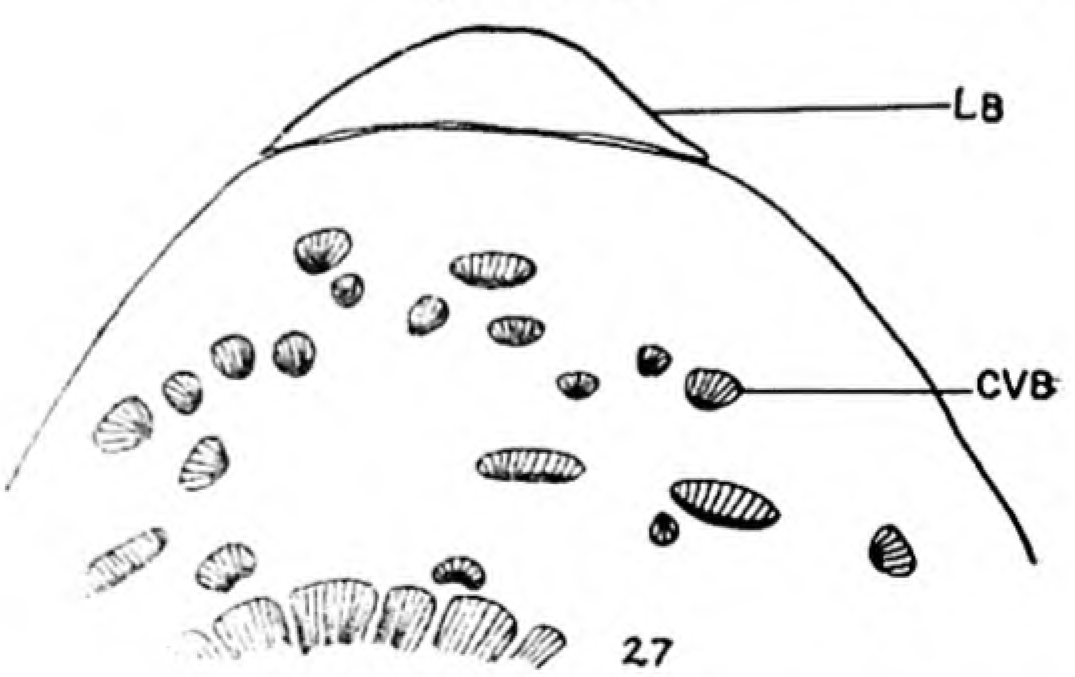

23

CVB
PX
CBO
SX
PRX
IPD

[illegible] sh[illegible] surro[illegible] cortical [illegible] x 3. Fig. 25 [illegible] origin of cortical b[illegible] Concentric and co[illegible] bundles having protoxy[illegible] mesarch, x 12. (Partially [illegible]

CBO, originating co[illegible] cortical vascular bundle; [illegible] peri-derm; *PX*, protoxylem; *PRX*, [illegible] xylem; *SX*, manoxylic secondary xylem





27

[illegible] 26, 27. *Purioxylon jurassica* gen. et sp. nov. Fig. 26. T. s. at the D-D′ level (cf. Plate Fig. 1) showing origin of an internal periderm in an axillary bud base and the surrounding cortical bundles, x 12. Fig. 27. T. s. same, showing the presence of a large number of collateral cortical bundles in the portion of cortex adjacent to the leaf base, x 12. (Partially Camera lucida sketches)

CVB, cortical vascular bundle; *PX*, Protoxylem; *SX*, manoxylic secondary xylem; *IPD*, internal periderm; *PDB*, periderm of axillary bud base; *LB*. leaf base.

bundles from the vascular zone are also seen. These are endarch in the beginning but later on may become exarch or mesarch (Fig. 13). Secondary xylem of the cortical bundles is not differentiated into growth rings and the tracheids are arranged in rows (Fig. 14). Xylem parenchyma are absent.

D—D′ (Fig. 24).—This section shows the anatomy of the stem in a transverse section passing through the base of an axillary bud. In the region of bud base, origin of an internal periderm is seen, which is not connected in any way with the internal periderm of the stem and arise *de novo* (Fig. 26). This periderm is made up of rectangular cells which are easily distinguished from the cells of the cortex. A number of cortical bundles are supplied to an axillary bud. In this slide the outer cortical bundles are arranged more or less, in rows (Fig. 25), particularly on either side of the bud base (Fig. 26). Cortical bundles are concentric or collateral, mesarch or endarch, and are provided with picnoxylic secondary xylem (Fig. 5). In this section the tissues of the vascular zone are comparatively better preserved and individuality of collateral bundles forming this zone can be seen (Figs. 6, 7). Each bundle ends on the outerside in an unpreserved area representing the phloem (Fig. 8). Origin of cortical bundles from the vascular zone is also seen in the slide (Fig. 9). One of the concentric type of cortical bundle is seen dividing and forming new bundles (Figs. 5, 25).

COMPARISON

The present material resembles in certain anatomical characters with the Cycads on the one hand and with the members of the group Pentoxyleae on the other hand. While some of its structures are peculiar of its own. Comparison of the new genus has also been made with the recently described stem genera from the Rajmahal Hills, for example *Sewardioxylon sahnii* Gupta (1960, 1971) and *Guptioxylon amarjolense* Sharma (1969). For detailed comparison see table 1.

DISCUSSION

Medullosan stem anatomy is considered to be similar with that of the Pentoxyleae (Sahni, 1948; Delevoryas, 1955; Stewart and Delevoryas 1956; Mittre 1957; Sharma 1969) on the grounds that in both these types of plants, vascular bundles forming the main stele are either concentric or endocentric having mesarch protoxylem points. But unlike the former medullary bundles are not found in the latter and so the group Pentoxyleae could not be correlated directly with the Medulloseae. A recently discovered stem from the Rajmahal Hills, *Guptioxylon endocentrica* Sharma (1971 in press) has bridged this gap. In this stem vascular bundles of the main stele are sharply endocentric resembling *Pentoxylon* while the presence of pith bundles shows its relationship with the *Medullosa*. An another species of *Guptioxylon*, *G. amarjolense* Sharma (1969a) possesses, besides, the medullary bundles, an internal periderm in the peripheral portion of pith. This feature is neither found in Pentoxyleae nor in Medulloseae, but is seen in the material of the new genus described in the present paper. These two types of stems also resemble in the structure, and nature of cortical bundles in having picnoxylic secondary xylem. Thus the

TABLE I

SHOWING COMPARISON OF *PURIOXYLON JURASSICA* GEN. ET SP. NOV. WITH ALLIED FOSSILS AS WELL AS LIVING PLANTS.

S. No.	Species	Reference	Horizon	Outer surface	Cortex	Pith	Vascular zone	Cortical bundles
1.	*Medullosa stellata*	Seward 1917	Permion	Close, spiral, rhomboid leaf bases	With mucilage canals	With concentric bundles each having its own secondary xylem	Siphonostelic with mesarch protoxylem points	Concentric or collateral with secondary xylem
2.	*Rhexoxylon piatnitzkyi*	Archangelsky and Brett 1961	Triassic	Close, spiral rhomboid leaf bases, branch scars circular with scale leaves	With cysts	With isolated xylem strands and cysts	Numerous bundles in a ring each having secondary growth on cpx as well as cfx sides	Collateral without secondary xylem
3.	*Guptioxylon amarjolense*	Sharma 1969	Jurassic	More or less smooth	With sclerotic nests	With concentric and collateral bundles each having its own secondary xylem and internal periderm present	Four concentric bundles each having its own picnoxylic secondary xylem	Concentric and collateral with its own picnoxylic secondary xylem
4.	*Guptioxylon endocentrica*	Sharma (1971 in press)	Jurassic	Faint rhomboid markings	With sclerotic nests	Concentric and collateral bundles each having its own picnoxylic secondary xylem	Six sharply endocentric bundles, each having its own picnoxylic secondary xylem	Collateral with picnoxylic secondary xylem
5.	*Pentoxylon sahnii*	Srivastava 1946	Jurassic	Old stems smooth, dwarf shoots—close, spiral, rhomboid leaf bases	With sclerotic nests	With sclerotic nests	5-6, sharply, endocentric bundles each having its own secondary xylem	Collateral with picnoxylic secondary xylem
6.	*Sewardioxylon sahnii*	Gupta 1971	Jurassic	Faint rhomboid markings	Without mucilage canals and sclerotic nests	Concentric or collateral bundles without secondary xylem	Two rings of collateral bundles, outer endarch inner exarch; manoxylic secondary xylem	Collateral without secondary xylem
7.	*Zamia floridina*	Greguss 1968	Living	Close, spiral; rhomboid leaf bases	With mucilage canals	With mucilage canals	Collateral, endarch bundles with manoxylic secondary xylem	Collateral without secondary xylem
8.	*Purioxylon jurassica* gen. et sp. nov	Sharma	Jurassic	Sparse, spiral, crescent shaped each with an axillary bud	With mucilage canal in peripheral portion	Unpreserved with an internal periderm	Numerous, collateral, conjoint, endarch bundles with manoxylic secondary xylem	Concentric or collateral with picnoxylic secondary xylem. Protoxylem exarch, endarch or mesarch

new genus can be indirectly correlated with the Pentoxyleae on the one hand and the Medulloseae on the other hand. But in the structure of the vascular zone, the present new genus differs from Pentoxyleae, Medulloseae, as well as from the stem genus *Guptioxylon* and in this respect it resembles the living Cycads, particularly those having monostelic and manoxylic vascular organization e.g. *Zamia, Stangeria*, etc. (Greguss 1968). But unlike the latter, the cortical bundles are provided with picnoxylic secondary xylem in the new genus, and position of protoxylem points vary in these bundles from endarch to mesarch or even exarch. Presence of secondary xylem in the cortical bundles (Leaf traces) is supposed to be a primitive feature (Stewart and Delevoryas 1956) and thus it may be concluded that the present genus shows an advancement over Medulloseae and Pentoxyleae in the nature of the vascular cylinder but the cortical bundles are still possessing conservative characters similar to those found in *Medullosa*, *Guptioxylon* and *Pentoxylon*.

The new genus described in the present paper differs from all the plants with which it has been compared in table 1, in the external morphological features. In the majority of Cycadophytes including Pteridosperms, Cycads. Benntitales and the Pentoxyleae (dwarf shoot), the outer surface of stem is provided with closely placed and spirally arranged, rhomboid leaf bases. However, in the old shoots of *Pentoxylon* and *Guptioxylon*, outer surfaces of stems are either smooth or only few leaf bases are present. In none of these types, axillary buds are present in the axil of leaf bases unlike the new genus. This character is peculiar of *Purioxylon jurassica* and for the present, it can not be correlated with any of the known living or fossil Cycadophyte. Similarly, the combination of anatomical characters in the new genus are very peculiar, possessing both advanced as well as, primitive features. However, a line of evolution may be suggested from Medulloseae to the Cycadaceae through the intermediate forms like *Guptioxylon amarjolense* and *Purioxylon jurassica*.

REFERENCES

ARCHANGELSKY, S., AND D. W. BRETT, 1961. Studies on the Triassic fossil plants from Argentina. 1. *Rhexoxylon* from Iscigualasto Formation. *Philos. Trans. R. Soc. London*. B. **244:** (706) : 1-10.

DELEVORYAS, T. 1955. The medulloseae, their structure and relationships. *Palaeontographica* B. **97:** 114-167.

GREGUSS, P. 1968. *Xylotomy of the Living Cyacads with a Description of Their Leaves and Epidermis*. Akademiai Kiado, Budapest.

GUPTA, K. M. 1960. On the anatomy of a new cycadean type of stem *Sewardioxylon sahnii* gen. et sp. nov. from the Jurassic of Rajmahal Hills Bihar. *Proc. 47th Indian Sci. Congr.* 428.

——. 1971. Investigations on the Jurassic flora of Rajmahal Hills, India. 9. On the structure and affinities of *Sewrdioxylon sahnii* gen. et sp. nov. Gupta, a petrified cycadean wood from the Rajmahal Hills, India. *Palaeontographica* **B. 131:** 160-166.

MITTRE, V. 1957. Studies on the fossil flora of Nipania (Rajmahal Series), India. Pentoxyleae. *Palaeobotanist* **6:** 29-46.

SAHNI, B. 1948. The Pentoxyleae, a new group of Jurassic gymnosperms from the Rajmahal Hills, Indian. *Bot. Gaz.* **110:** 47-80.

SHARMA, B. D. 1967. Investigations on the Jurassic flora of Rajmahal Hills, India. 3. A review of the genus *Ptilophyllum* of morris with description of two new species from Amarjola in the Rajmahal Hills, India. *Palaeontographica* B. **120:** 139-150.

——. 1968. 5. Epidermal studies in the bracts of two new species of *Williamsonia*. *W. guptai* and *W. amarjolense*. *Acta. Bot. hung.* **14:** 373-383.

——. 1969a. *Guptioxylon amarjolense* gen. et sp. nov. from Amarjola in the Rajmahal Hills, Indian. *Palaeontographica* B. **126:** 145-153.

——. 1969b. On Pentoxyleae remains from Amarjola in the Rajmahal Hills, India. *Ameghiniana* **6:** 50-56.

SHARMA, B. D. 1971. Further observations on *Sewardioxylon sahnii* Gupta collected from the Middle Jurassic of Rajmahal Hills, India. *Acta. Bot. Neerl.* **20:** 475-480.

——. *Guptioxylon endocentrica* sp. nov. from the Jurassic of Amarjola in the Rajmahal Hills, India. *Bull. Soc. Linn. Lyon.* (In press.)

SRIVASTAVA, B. P. 1946. Silicified plant remains from the Rajmahal Series of India. *Proc. Natn. Acad. Sci. India.* **15:** 190-211.

STEWART, W. N. AND T. DELEVORYAS. 1956. Medullosan Petridosperms. *Bot. Rev.* **22:** 45-80.

Adv. Pl. Morph. 1972: 243–250

ON THE PHENOMENON OF CAULIFLORY IN ANGIOSPERMS*

BY Y. P. S. PUNDIR

Deptt. of Botany, D. B. S. College, Dehradun, India

ABSTRACT

The phenomenon of cauliflory is largely met in some tropical trees. It is little investigated morphologically. Schimper (1898) belived that cauliflory is due to exogenous surface buds. But subsequently it was shown by several workers that cauliflorous shoots in large number of plants develop regularly from endogenous buds. At present therefore, there are three possible behaviours concerning cauliflory. (1) Cauliflory solely due to exogenous buds. (2) Cauliflory solely due to endogenous buds. (3) Cauliflory due to both exogenous and endogenous buds on the same plant. On the basis of development of cauliflorous shoots Lent (1966) has recognized two types of cauliflory—"adventative cauliflory" and "axillary cauliflory." Some workers believe that cauliflory is an adaptation phenomenon that has resulted through natural selection and cauliflorous plants are of archic nature.

Cauliflory (Caulis=stalk or stem and flos or floris=flower i.e., production of flowers from the old wood, main trunk and old branches) is an interesting phenomenon met with largely in tropical trees that, besides, the usual type of flowering on normal shoots, bear flowers on the old branches and trunks. Although many cauliflorous shrubs and trees are economically important, they have received little attention of morphologists and physiologists. Thus our knowledge of this interesting phenomenon is rather fragmentary and there is some difference of opinion on some points.

Earlier work on cauliflory has been reviewed by Lopriore (1907) and in present paper an attempt will be made to bring this up to date.

Schimper (1898) listed a number of cauliflorous plants from Java and on the basis of place of origin of cauliflorous shoots he recognised three types of cauliflory. (a) Cauliflory on the main stem, (b) Cauliflory on both the branches and main stem, (c) Cauliflory on the branches only.

He brought forward an explanation of this peculiar mode of flowering and ascribed it to dormant exogenous buds which were previously formed in the axils of leaves. These buds remain dormant for many years on leafless thick branches and

---

*The intention to prepare this article is that the little knowledge which we have about Indian cauliflorous plants is the result of the work done under Prof. V. Puri's guidance at his laboratory.

I am highly thankful to Prof. V. Puri for going through the manuscript critically and for valuable suggestions. I am also thankful to Dr Y. S. Murty and Dr V. Singh for their help inv arious ways.

trunk and ultimately "break through" the cortex and produce the flowering shoots. As to cauliflorous plants being commonly tropical, Schimper wrote that cauliflory "is owing to the weaker development or less toughness of bark. This opinion is supported by the fact that in dry districts where the bark is considerably developed and rich in fibres, cauliflory is rare."

Schimper's view that all cauliflorous shoots arise from exogenous buds was supported by facts available then. But subsequently several examples of cauliflorous shoots developing regularly from endogenous buds formed in a complex manner in deeper layers of bark were brought to light(Thompson 1952; Sharma 1959; Pundir 1965, 1971). At present therefore, there are three possible behaviours concerning cauliflory: (i) Flowering solely through exogenous buds, (ii) Flowering solely through endogenous buds, (iii) Flowering both through exogenous and endogenous buds.

Cauliflory due to exogenous buds.— Thompson (1944, 1946, 1949, 1951, 1952) has studied the problem in detail in several species and has made valuable contributions to the subject. In *Cercis siliquastrum* (Leguminosae) he (Thompson 1946) has shown that cauliflory is due to the presence of accessory buds formed exogenously by axillary meristems. In this plant all the axillary buds are consumed in foliar shoot formation whereas some accessory buds remain dormant and cause delayed primary flowering upon mature leafless portions of the twigs. The stumps of primary inflorescences also carry exogenous buds. However, the development of these buds is delayed. The exogenous buds give rise to further inflorescences. The yearly repetition of flower production from old sites of initial flowering leads to a massing of stumps which go on producing exogenous buds developing into inflorescences upon sturdy leafless branches and more massive trunk.

In *Forsythia suspensa* (Oleaceae) in contrast to Cercis *siliquastrum,* the primary axillary buds and accessory secondary buds remain dormant for one year on leafless shoots and with advance in secondary growth they are mainly cork-clad and stand in a linear succession immediately above the leaf scars. During the ensuing spring the primary bud quickly gives rise to an inflorescence which now dominates. The accessory and subjacent accessory buds of the dominant bud do not develop further until it dies out. With the shedding of dominant inflorescence, accessory buds in flowering season quickly form flowering shoots. According to Thompson (1946) *Forsythia suspensa* attains only a condition of ramiflory.

Pleiocarpa mutica (Apocynaceae) exhibits a more complicated mode of flowering (Thompson, 1949). This shrub first flowers by simple crown-borne cymes. Each phase of vegetative growth is closed by a large leaf subtending a row of buds, which under certain conditions show delayed dormancy. Some buds in upper most regions may produce foliar shoots, but in older parts upper most accessory buds form inflorescences. The inflorescence formation does not involve all buds in one season and after the abscission of inflorescence some buds are left on the stumps. When crown branching has mainly run its course, dormant buds beneath the crown break into flowering and this process extends to still lower buds. Thus this becomes a case of long ending flowering state with widening flowering fields (Fig. 3.).

In *Swartzia* (Leguminosae) flowering patterns vary in different species and S. *pinnata* has been studied in detail by Thompson (1951). According to him plants are thought to flower at the age of 15 years. At the end of season's growth the twig ends in a slender shoot and between this and last expanded leaf is formed a series of buds which extend down to a meristem filled pit in the leaf axil. The behaviour of these buds is variable. Sometimes an upper most bud may grow out and cause forking of the branch or may persist and later form an inflorescence. Similarly the lower buds of a series may also develop into inflorescences. It is very important as regards this plant that the lower bracts of the receme inflorescence subtend buds which remain somatic at first but after the abscission of the inflorescence above this level, these somatic buds are left on the stumps. Later these buds form inflorescences, this sequence is repeated and at last the stumps build up a burr which has a capacity for indefinite flowering.

Initiation of cauliflorous inflorescences in *Theobroma cacao* (Sterculiaceae) has attracted the attention of many workers and there appears to be some difference of opinions which have been briefly reviewed by Lent (1966). With these views in mind, Lent (1966) has attacked the problem of cauliflory in this plant in two ways: (i) by tracing the vascular traces of a flowering shoot on a mature branch and trunk, back to their source and (ii) by following the development of axillary buds to their ultimate stage. *Theobroma cacao,* according to him, has at least two buds in the axils of all the leaves. One is the principal axillary bud and the second a subordinate bud* of the first (Fig. 2). The subordinate bud remains dormant for varying periods in the bark and finally form an inflorescence on mature branches and trunk at old axillary positions. The axillary pits also may have reserve subordinate meristems which may form additional subordinate buds if required.

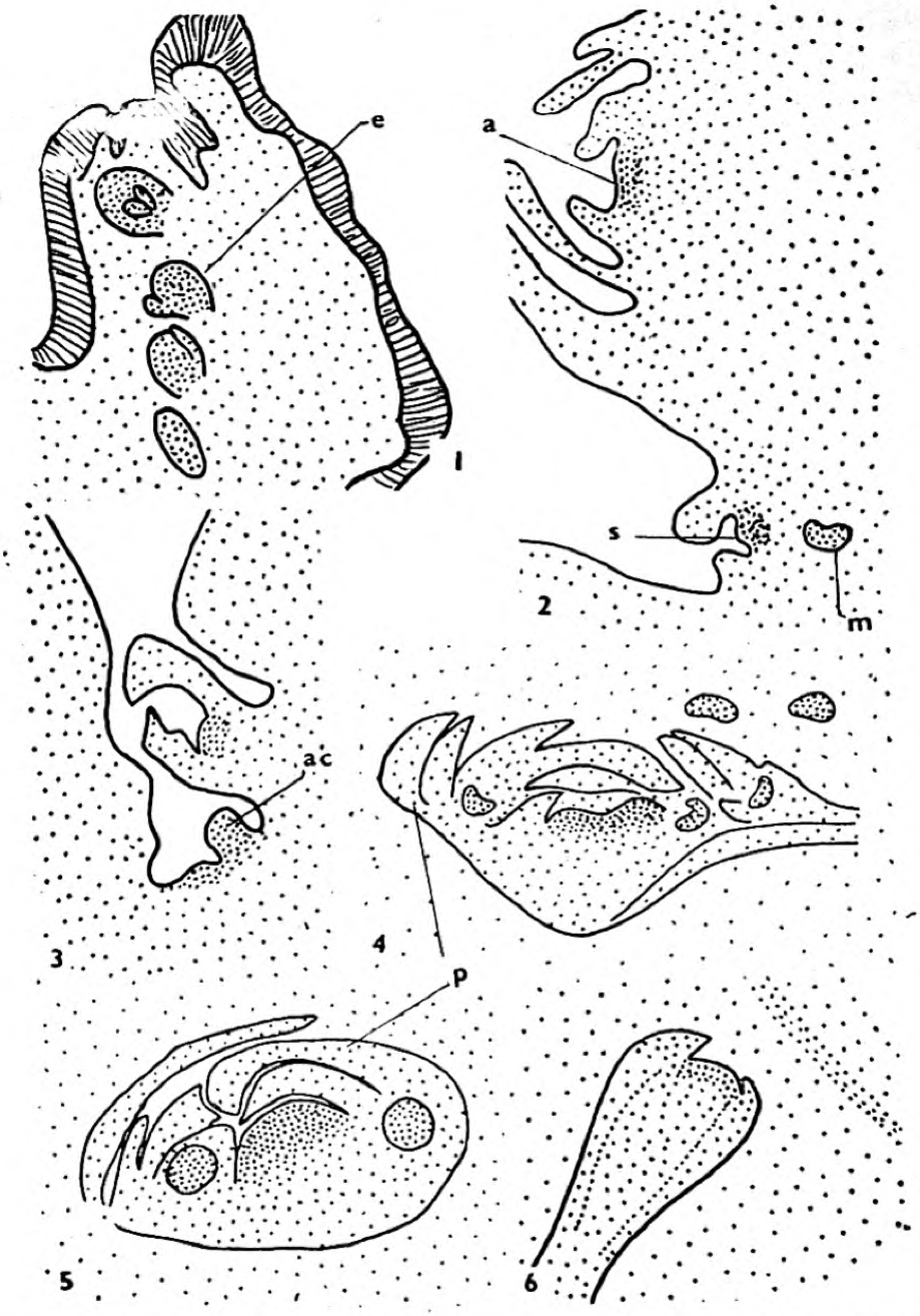
Cauliflory due to endogenous buds.—We know of a few species which exclusively flower due to endogenous buds produced in the bark. Thompson (1952) has studied the cauliflory of *Couroupita quianensis* (Lecythidaceae) in great detail and found that this plant flowers completely by endogenous buds which form inflorescences. Here all the axillary buds are consumed in the formation of foliar shoots or they die in the bark. The flowering shoot develops from a compound bud developing *de novo* from meristems arising in cortex beneath the unbroken cork. Each compound bud has a large round central bud with prophylls and numerous concave smaller buds arranged around it (Fig. 4). The whole system of compound bud is pushed upwards by the intercalary growth in the body of the large bud and it breaks through the cortex to the surface. When the central bud is exposed, the prophylls expand and the lateral buds are also exposed. Once the flowering shoot bud is exposed all the lateral organs develop as product of the apex.

The present author (Pundir 1971) has also observed almost a similar condition in *Ficus cunia*. In this plant the axillary buds develop into foliar shoots or they die. When the plant attains an age of 8-10 years, first cauliflorous shoots develop at the base of the trunk from endogenous buds which are formed *de novo* in the deeper layers of bark. With advancing age the flowering is shifted to the distal parts of the trunk and the thick limbs.

Cauliflorous plants with exogenous and endogenous flowering.—These plants in one phase of life flower by exogenous buds which may be primary axillary or accessory buds, while in other phase of life the flowering shoots arise from endogenous buds. In *Ceratonia siliqua* (*Leguminosae*)' for in-

---

* Lent (1966) objects to the use of such terms as accessory buds or secondary buds and purports subordinate bud as a suitable term.



FIGS. 1-6. Fig. 1. L.s. showing endogenous buds of *Artocarpus heterophyllus*. Fig. 2. L. s. of a node of *Theobroma cacao* showing an axillary bud, subordinate bud and subordinate meristem. Fig. 3. L. s. showing accessory buds of *Pleiocarpa mutica*. Figs. 4, 5. Compound endogenous buds with prophylls, in *Couroupita guianensis* and *Ficus glomerata* respectively. Fig. 6. L. s. showing endogenous bud of *Ceratonia siliqua*. (Fig. 1, after Sharma 1959; Fig. 2, after Lent 1966; Figs. 3, 4 and 6, after Thompson 1944, 1949, 1952; Fig. 5, after Pundir 1965).

a, axillary bud; ac, accessory bud; e, endogenous bud; m, subordinate meristem; p, prophylls; s, subordinate bud.

stance (Thompson 1944) solitary axillary exogenous buds are produced on all current shoots. Such buds quickly give rise to foliar shoots or primary inflorescences in the axils of subtending leaves. Later the stumps of these primary inflorescences come gradually to assume positions well removed from their related leaf scars, due to the growth activities of the axillary meristems. These meristems will bear neither accessory exogenous buds nor any superficial organs of any kind throughout their later part of life. However, one major result of their prolonged activity is the broadening and protrusion of the stumps, so that the leafless cork-clad portions of all branches become dotted with prominent wens. It is deep within these wens that the meristems form endogenous buds which give rise to the later inflorescences of the plant (Fig. 6). Thus Thompson (1944) concludes that the cauliflory of *Ceratonia siliqua* is "due mainly to seasonal repetition of this endogeny and a rhythmic protrusion of wen borne inflorescences in crowded groups along the course of aging branches".

In a later communication Thompson (1946) has pointed out that the members of Lecythidean Myrtaceae also first flower by exogenous buds and later display cauliflory broadly like that of *Ceratonia siliqua*, by endogenous buds.

In *Artrocarpus heterophyllus* (Moraceae) Sharma (1959) has observed that buds developing on normal terminal shoots fail to produce inflorescences. But those developing on older branches in basipetal fashion are dormant buds and may produce small sized inflorescences which rarely produce very small sized fruits. Buds developing into fertile flowering shoots on still older limbs and trunk 'appear' to be exogenous dormant buds. Soon after these areas become raised up and cork clad and in the next flowering season there starts the *de novo* production of endogenous secondary buds at these sites (Fig. 1).

More recently Pundir (1965, 1971) has studied the origin of cauliflorous shoots in *Ficus glomerata* and *F. pomifera* (Moraceae). He has shown that *F. glomerata* shows a series of transition from exogenous to endogenous conditions. The flowering in these trees starts when they are about 7-8 years old. The first inflorescences develop from some of the axillary buds of the upper most leaves. Subsequently 2-3 years old branches also develop inflorescences in axillary positions and finally the inflorescences are produced on old branches and trunk. In old trees a few axillary buds on terminal branches produce foliar shoots and some die off while others become dormant. With advancing age these dormant buds get surrounded by cork. When a branch is 3-5 cm thick, some dormant buds on it become active and give rise to flowering shoots. But at the same time there starts *de novo* production of new compound buds in large number from the deeper layers of bark (Fig. 5). The buds develop from meristems which are organized in close neighbourhood of dormant buds or near by the stumps of shriveled buds, at various depths in the cortex. A few compound buds protrude above the surface and finally one develops into a flowering shoot while the remaining ones die off. This flowering shoot persists for several years and does not check the formation of new buds in the bark although these never develop further during its life time. *Ficus pomifera* also shows an almost similar condition.

General views on cauliflory.—Some authors hold the view that cauliflory is an adaptation phenomenon that has resulted through natural selection. Ducke (1922), Pijl (1941, 1956) have described cauliflory as an adaptation to bats. According to the latter Cauliflory of many species which are chiropeterophilous and chiropeterochorous is an ecological adaptation between themselves and bats. Corner (1949), on the other hand, in his Durian Theory of the origin of modern tree speaks that in late desent cauliflorous plants have become so adapted in their mode of flowering to low flying insects which are constant visitors to such plants. And cauliflory is a condition forced upon leptocaul trees (plants with slender twigs and hard wood) with usually applanate foliage by the retention of old massive forms of flower and fruit. Thus according to him cauliflory in modern trees is a remnant of morphological structures of primitive plants. Scaramella (1932) also believes that cauliflorous plants are of archic nature.

That cauliflory is a primitive character may be true for some cauliflorous plants but many cauliflorous plants appear to be far from being primitive. Pijl (1956) also believes that cauliflorous chiropeterophilous plants like *Kigelia Crescentis*, *Parmentera*, *Eugenia*, *Amphitocera*, *Duiro*, *Mucuna* and many Sapotaceae cannot be regarded as primitive. Further anatomical investigations also support that cauliflorous plants are not primitive and cauliflorous state is a secondary character which is obtained in various ways and is of quite complex nature (Thompson 1951).

According to Scaramella (1932) a disturbance in the supply of water and nutrient salts is responsible in bringing about the cauliflorous state by changing the ordinary vegetative bud in the bark to flower bud. His assumption was largely based on the fact that the cauliflorous plants are abundantly found in tropical rain forests where transpiration is slight.

But this view needs confirmation and does not offer a satisfactory explanation. As trees growing in dry climatic conditions show more transpiration and little water can be retained in their stems so that under such conditions these plants in course of time would have shifted their flowering from trunks and limbs to terminal branches. But such a condition has never been observed any where.

Fries (1949) has studied the cauliflory in some South American species of Annonaceae. Its taxonomical value is considered to be small by him.

It is quite apparent that the study of cauliflory should be extended to more genera which show still more peculiar ways of flowering. We do not know why or how the axillary buds become dormant and after some time which is variable even for different buds on the same plant, these buds are activated to form inflorescences. Further we are not aware about its morphophysiological significance and taxonomical value. But atleast one thing is certain that cauliflorous shoots develop in two ways. (i) The inflorescence develops from an endogenously formed bud initiated by an off mass of meristematic cells in the deeper layers of tissue of the older branches or trunk. In such cases the axillary buds die off or exhaust in the production of foliar shoots. (ii) The inflorescence develops from a principal axillary or a subordinate surface bud which remain dormant in an axillary pit almost covered by bark for variable periods. The axillary pit also may contain reserve axillary meristems which may assume the function if the principal bud is destroyed.

These developmental lines have led Lent (1966) to conclude that separate terms 'adventitive cauliflory' and 'axillary cauliflory' should be used. But the application of these terms may cause some confusion as when they are applied on plants which simultaneously produce cauliflorous shoots by axillary buds or axillary dormant buds and endogenous buds.

TABLE I

SITE AND MODE OF ORIGIN OF CAULIFLOROUS INFLORESCENCES IN SOME KNOWN PLANTS

Name of the Plant	Mode of origin			Site of origin of inflorescence
	Exogenous		Endogenous	
	Principal axillary Bud	Accessory or subordinate bud		
1. *Cercis siliquastrum*	Foliar shoots only	develop from meristems and form inflorescences	×	Flowering from stumps.
2. *Forsiythia suspensa*	Foliar shoots, some become dormant and form primary inflorescence	dormant and form Sec. inflorescence, after shedding of primary	×	Twigs.
3. *Pleiocarpa mutica*	Foliar shoots or inflorescences	Inflorescences	×	axillary; and on old branches:
4. *Swartzia pinnata*	Foliar shoots	Inflorescences	×	old branches.
5. *Theobroma cacao*	Foliar shoots	Inflorescences	×	old branches.
6. *Couroupita guianensis*	Foliar shoots or die		Compound endogenous buds form all inflorescence	on trunk and thick limbs
7. *Ficus cunia*	Foliar shoots or die		all inflorescences	on trunk limbs and trunks.
8. *Ceratonia siliqua*	Foliar shoots and Primary inflorescences		Secondary inflorescences	Primary inflorscences are axillary and secondary inflorescences cauline.
9. *Artocarpus heterophyllus*	Foliar shoots, basal dormant buds form sterile inflorescences		Endogenous buds form fertile inflorescences	Trunk and thick branches
10. *Ficus glomerta*	Foliar shoots, dormant buds form inflorescences		Endogenous compound buds form inflorescences	Young plants on axillary position and old plant on trunk and thick limbs.
11. *Ficus pomifera*	Foliar shoots; dormant buds ?		Endogenous	Thick branches and trunk.

REFERENCES

CORNER, E. J. H. 1949. The durian theory or the origin of the modern tree. *Ann. Bot. N. S.* **13:** 367-414.

DUCKE, A. 1922. Plantes nouvelles ou peu connues de la région amazonienne. Ze Partie. Archivos do Jordim. Botanico do Rio de Janiero, 3.

FRIES, ROB. E. 1949. Sobre la caulifloria en. la familia de las Anonaceas (About the cauliflory in the family Annonaceae). *Lilloa* **16:** 251-261.

LENT, ROY. 1966. The origin of cauliflorous inflorescence of *Theobroma cacao*. *Turrialba* **16:** 352-358.

LOPRIORE, G. 1907. *Die Cauliflorie Verlag von Gustav Fischer*. Jena.

PIJL, L VAN DER, 1941. Flagelliflory and cauliflory as adaptations to bats in *Mucuna* and other plants. *Ann. Bot. Gard. Buitenzorg.* **51:** 83-93.

——. 1956. Remarks on pollination by bats in the genera *Freycinetia, Duabanga* and *Haplophragma* and on Chiropterophily in general. *Acta. Bot. Neerl.* **5:** 135-144.

PUNDIR, Y. P. S. 1965. *Morphological Studies in Ficus glomerata with Special Reference to Cauliflory*. Ph. D. Thesis, Agra University, Agra.

——. 1971. Cauliflory in *Ficus cunia* and *F. pomifera* (In press).

SCARAMELLA, P. 1932. La Caulifloria nelle piu recenti vedute. *Malpighia* **22:** 1-11.

SCHIMPER, A. F. W. 1898. *Plant Geography upon a Physiological Basis*. English translation by W. R. Fisher, Revised by P. Groom and I. B. Balfour, 1960. Herts.

SHARMA, M. R. 1959. *Morphological and Anatomical Investigations in Artocarpus*. Forst. Ph. D. Thesis Agra Univ. Agra.

THOMPSON, J. MCLEAN. 1944. Towards a modern physiological interpretation of flowering. *Proc. Linn. Soc. Lond.* **156:** 46-69.

——. 1946. The study of plant-behaviourism; a common meeting-ground for those engaged in botanical inquiry. *Proc. Linn. Soc. Lond.* **157:** 72-91.

——. 1949. A contribution to our knowledge of the Apocynaceae with special reference to *Pleiocarpa mutica*. *Proc. Linn. Soc. Lond.* **161:** 12-26.

——. 1951. A further contribution to our knowledge of cauliflorous plants (with special reference to *Swartzia pinnata* Willd.) *Proc. Linn. Soc. Lond.* **162:** 212-222.

——. 1952. A further contribution to our knowledge of cauliflorous plants with special reference to the cannon ball tree (*Couroupita guianensis* Aubl.) *Proc. Linn. Soc. Lond.* **163:** 233-250.

Adv. Pl. Morph. 1972 : 251-254.

SUR LA CLASSIFICATION DES FRUITS (ABOUT THE CLASSIFICATION OF FRUITS)

By Jean-Louis Guignard

Laboratoire de Morphologie Végétale de la Faculté de Pharmacie de Paris-Sud,
92—Chatenay-Malabry

Abstract

There are two great families of fruits, this of indehiscent and uniseminated fruits and that of dehiscent and multiseminated fruits; that division corresponds to a physiological and ecological reality: dispersion of the seeds at the stage of the fruit or scattering at the stage of the grain ; for the two series, the succulence is a character obtained afterwards, and the classification in dry and fleshy fruit does not correspond to a natural division.

La classification des fruits en secs et charnus est fort ancienne: elle est simple, parlante, concrète. Nous pensons cependant qu'elle ne correspond pas à une division naturelle.

Pour nous il existe deux grandes varéités de fruit: les indéhiscents et les déhiscents. Le premier groupe comprend les akènes (caryopses, samares, nucules....) et les drupes ainsi que leurs formes dérivées: polyakènes, drupes multispermes; le second, les fruits secs déhiscents (capsules, follicules, gousses, siliques....) et les baies.

Chez les *fruits indéhiscents* les parois de l'ovaire ou plus rarement celles de chacune des loges ovariennes se lignifient et enferment une graine, rarement deux-ou plus: les fruits indéhiscents sont très généralement uniséminés. Cette unicité se comprend: l'enveloppe du fruit, très résistante, ne sera détruite qu'une fois enfouie dans le sol, sous l'influence des intempéries et des attaques des microorganismes, ce qui ne saurait assurer la dispersion de plusieurs graines; celles-ci se trouveraient disposées côte a côte et se gènerait lors de la germination.

La présence d'un tegument séminal mince et très généralement dépourvu d'annexe s'explique également puisque la protection de la graine est, en fait, asurée par la paroi sclérifiée du fruit; remarquons de même que la dispersion des graines est alors réalisée au niveau du fruit et ce dernier pourra acquérir divers ornements favorisant la diasporie.

Primitivement, toute la paroi de l'ovaire se dessèche et se lignifie : on a un fruit sec, indéhiscent et uniséminé, c'est-a-dire un *akène* (de *a* privatif et du grec, *kainen* ouvrir) dont la dispersion est très souvent favorisée par des crochets qui se fixent à la toison des animaux ou par des ailes membraneuses et des aigrettes qui permettent au vent de les entrainer.

Dans un stade plus évolué, la dispersion est assurée notamment grâce a la gourmandise des animaux : pour cela seule la partie interne de l'ovaire se sclérifie—le noyau ou endocarpe—tandis que les parties externes (péricarpe et mésocarpe) deviennent molles et plus ou moins succulentes : on a une *drupe*, fruit charnu, indéhiscent et très généralement uniséminé. Les drupes ne sont ainsi que des akènes dont les parties externes sont devenues charnues.

Akènes et drupes bien qu'uniséminés dérivent d'ovaires simples ou composés multiovulés : il y a avortement de tous les ovules sauf un et éventuellement de toutes les loges moins une. Par exemple les fruits a noyau des Rosacées proviennent généralement d'ovaires biovulés dont un ovule avorte; exceptionnellement les deux ovules sont fertiles et l'on trouvera deux graines dans chaque coque. Cependant, dans certains cas, plus exceptionnels, chaque loge se transforme individuellement en une enveloppe close et lignifiée (méricarpe): on aura des diakènes a partir d'ovaires biloculaires, des polyakènes chez les ovaires pluriloculaires. A maturité chaque akène s'individualise par délamination, plus ou moins complexe, des septums. Citons les diakènes des Ombellifères et des Rubiacées, les polyakènes ou schizocarpes des Malvacées. Un mécanisme voisin se retrouve chez quelques drupes dites polyspermes: chaque loge donne naissane à un noyau, lequel s'individualise ensuite par pourriture de la pulpe sucrée. Un bon exemple est donné par le fruit du café.

De façon générale on rencontre les fruits indéhiscents chez les espèces dont les carpelles restent indépendants c'est-à-dire chez les plantes a gynécée archaïque; toutefois les fruits indéhiscents dérivent parfois de fruits primitivement déhiscents et pluriséminés, devenus secondairement indéhiscents et par la-même uniséminés; c'est le cas de nombreuses Malvacées, de quelques Légumineuses, Crucifères. .

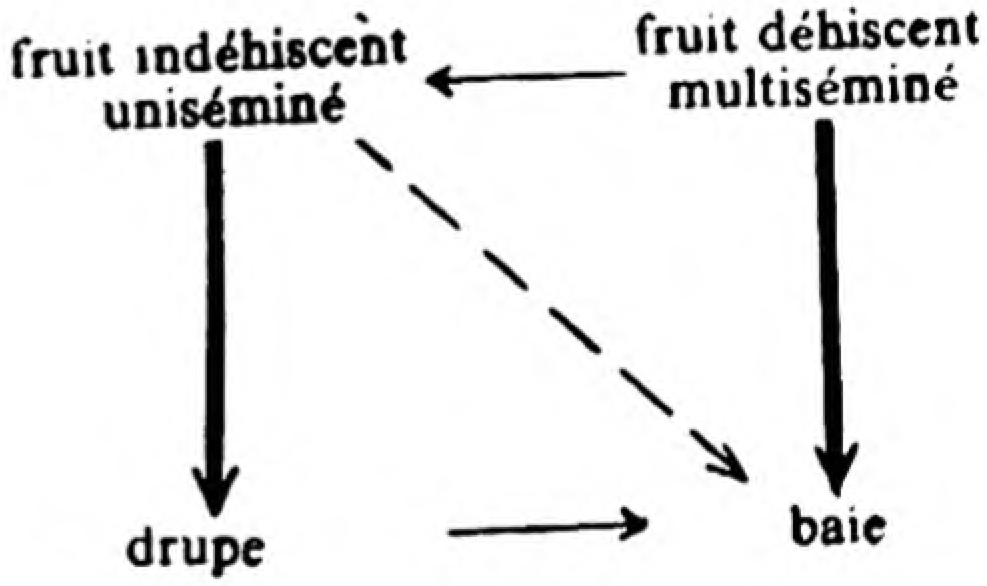
Le deuxiéme grand groupe de fruit comprend ceux qui sont *dèhiscents* et très gènèralement multispermes. Ces caractères vont de pair : s'il y a plusieurs graines dans le fruit, il faut, pour assurer à chacune d'elles une dissémination efficace, qu'elle s'échappe de façon prècoce, donque le fruit s'ouvre ou se dèsorganise facilement. La diasporie de l'espèce est ici réalisée au stade de la graine ce qui explique que les téguments séminaux soient épais, résistants et fréquemment pourvus d'annexes, arilles, arillodes, poils, aigrettes......

Les fruits pourvus de dispositif d'ouverture sont, à quelques exceptions très (fruit de la balsamine par exemple), nécessairement des fruits secs, les mécanismes de dehiscence exigent en effet des tissus déshydratés et plus ou moins sclérifiés que les variations hygroscopiques font "jouer" et finalement rompre aux zones de moindre résistance, fentes et pores. L'emplacement de ces zones de rupture est très variable. On distingue les ouvertures réalisées au niveau de la suture de la feuille carpellaire ou des carpelles, celles qui se produisent au niveau de la nervure dorsale des mêmes organes, celles qui ont lieu de part et d'autre des placentas ou encore de facon particulière comme celles qui dèlimitent un opercule au sommet du fruit. Ces modalités diverses d'ouverture et le fait que le fruit dérive d'un seul carpelle ou de plusieurs soudés entre eux permet de définir une grande variété de *fruits secs déhiscents*, capsules, follicules, gousses, siliques, pixides...bien connus des morphologistes.

Les baies, fruits charnus et très généralement multiséminés deivent également être considérés comme des fruits primitivement déhiscents, mais que l'évolution a rendu secondairement charnus. La succulence du fruit supprime tout mécanisme d'ouverture : les graines sont libérées par le pourrissement des parties molles qui les entourent ou se retrouvent, après passage par le tube digestif des animaux, dans leurs excréments; ce passage n'altère généralement pas les graines ce qui explique que les oiseaux qui se nourrissent souvent dans des lieux éloignés de leur nid puissent disséminer très efficacement certaines graines, comme celles du Gui.

La réalité de la division entre fruits indéhiscents et déhiscents est prouvée par le fait que, dans les limites d'un même taxon, on renventre soit des akènes et des drupes, soit des fruits secs déhiscents et des baies. En effet lorsqu'on assiste, dans un phylum, a l'acquisition de la succulence, le fruit est une drupe ou une baie suivant qu'il provient d' organes primitivement indéhiscents ou déhiscents : ainsi chez les Caryophyllacées caractérisés par un fruit capsulaire, le *Cucubalus* possède des baies; de même chez les Renonculacées les fruits de l' *Actae* sont bacciformes; par contre les méricarpes des Ombellales donnent naissance à des drupes chez les Lierres, les Cornouillers; les akènes des Fraisiers ont leur homologue dans les drupéoles des Framboisiers; les samares des Frênes correspondent aux drupes des Oliviers, les follicules des Lilas aux baies des Troènes, etc.

Des passages, comme dans toutes les structures vivantes, sont toutefois observables entre les deux grande groupes de fruits. C'est ainsi que chez tous les grands taxa caractérisés par des fruits multiséminés on observe dans quelques lignées evolutives une tendance à la diminution du nombre des graines dans le fruit; les baies qui deviennent monospermes, les silicules uni ou biséminées des Cruciféres, les gousses pauci-séminées des Papilionaccées sont de bons exemples. Dans le cas des fruits secs, ces derniers deviennent généralement indéhiscents (Kakilier, Sainfoin....) ; qu'ils acquièrent secondairement le caractère charnu et nous aurons un fruit drupoïde (Benjoin), alors que l'on attendait un fruit bacciforme. De même, chez les drupes, l'endocarpe peut régresser, devenir membraneux et disparaitre. Chez les pommes, les poires, les coques sont déjà minces et papyracées; ce ne sont plus des fruits à "noyau" mais a "pepins". A la limite, le fruit acquiert l'aspect d'une baie. Chez les akènes dont la paroi ne comporte pas de tissu scléreux lignifié, la transformation en une pulpe succulente est entière et l'on passe directement a une structure du type baie ; c'est ce qui se passe chez la Garance, plante du groupe des Rubiées caractérisé par des akenes à paroi cellulosique. Ce ne sont là que des cas extrèmes des exceptions qui confirment la règle.



En conclusion, il existe deux grandes familles de fruits, celle des fruits indéhiscents et uniséminés et celle des fruits déhiscents et multiséminés; cette division correspond à une réalité physiologique et écologique : dispersion des semences au stade du fruit ou dissémination au stade de la graine; la succulence est dans les deux séries, un caractère secondairement acquis.

Dés lors, la classification entre fruits secs et fruits charnus, bien que pratique, ne mérite pas d'être maintenu, tant par les morphologistes que par les phylogénistes.

Adv. Pl. Morph. 1972: 255–263.

POLLEN MORPHOLOGY AND PHYLOGENETIC CLASSIFICATION OF PRIMITIVE ANGIOSPERMS[1]

BY P. K. K. NAIR

National Botanic Gardens, Lucknow, India

ABSTRACT

Pollen morphology of primitive angiosperms has indicated that there are two primary dicot stocks namely the "Magnolian" stock (Magnoliaceae and its allies), and the "Ranalian" stock (Ranunculaceae and its allies), each stock being characterised by monocolpate and tricolpate pollen respectively. Comparing the various systems of angiosperms classification, the systems of Bessey and Cronquist conform with the facts of pollen phylogeny, with regard to primitive angiosperms. Of these, the system of Cronquist is more acceptable, because of the fact that he classified all the taxa of dicots with monocolpates (primitive pollen type) in the subclass Magnoliideae. It has been proposed that the two subclasses Ranunculiideae and Magnoliideae of Cronquist, should be considered to represent the two palynological stocks, and should be raised to higher taxa levels.

INTRODUCTION

The Darwinian era of botanical history is marked by a revolutionary drift in the trend of biological thought, caused by the genesis of phylogenetic ideas and its consequent application in studies of plant morphology, relationships and classification (see Wardlaw, 1952, 1968; Good, 1956; Mayr, 1963; Cronquist, 1968; Briggs and Walters, 1969; Duddington, 1969; Crowson, 1970; Manwell and Bakker, 1970; Harborne, 1970; Young and Watson, 1970; Margoliash and Fitsch, 1971; Stebbins, 1971). With the realisation that the plant body is an intricate interlink of diverse morphosystems, the understanding and analysis of evolution and phylogeny of any one plant form have become difficult. However, the reproductive structure (perhaps also including its ultimate units formed by pollen or spores) is recognised as the most conservative of all morphosystems in plants, which merits the consideration of floral organs in phylogenetic treatises and consequently, phylogenetic classifications reflect the conflict of concepts on the phylogeny of the flowers and of the floral organs primarily, and of other morphosystems secondarily.

The angiosperms constitute a group of plants that embody the climax of evolution of the various plant morphosystems. For example, the reproductive units (spores and pollen) alone reflect, to a fair degree, the trend of morphological evolution beginning with the Algae and culminating in the angiosperms (see Nair, 1970). The uncertain trends of spore morphological evolution in the Non-Archegoniatae (i.e., algae, fungi, lichens) ceased with the origin of the bryophytes (see also Erdtman, 1957, 1965, in Gunckel, 1969) in which a definite trend of evolution was set in motion which is in conformity with the orthodox method of judging evolutionary trends in terms of survival value of characters. This new trend was marked by the organisation of the fundamental aperture morphoform, namely the *trilete,* from which evolved two other morphoforms, namely the *monolete* and the *alete.* This trend of evolution continued to exist in the pteridophytes and in the gymnosperms. However, a marked shift from the general line of spore evolution is the origin of heterospory in the pteridophytes which is assumed to be the precursor of the seed habit in the phanerogams (see Sussax, in Cutter, 1966).

---

[1] An account of the preliminary observations on the subject was presented at the III International Conference on Palynology, at Novosibirsk, U. S. S. R. in July, 1971.

Considering the trends of morphological evolution of the Archegoniatae (bryophytes, pteridophytes and gymnosperms; see also, Mehra, 1968), it is reasonable to presume that the fundamental apertural morphoforms in the primitive angiosperms at least, must have been comprised of the *trilete* (trichotomocolpate), the *monolete* (monocolpate), and the *alete* (acolpate). Due to the woeful paucity of information on pollen fossils of the primitive angiosperms (Axelrod, 1970) there is an inevitable lack of factual fossil evidence to substantiate the above contention. However, a broad analysis of pollen morphology of the present-day angiosperms shows that the trilete-monolete-alete (trichotomocolpate-monocolpate-acolpate) complex is dominant in the monocots, and is the only prevalent type in a section of dicotyledones represented by the Magnoliaceae and its allies. In a comparative analysis of the various systems of angiosperm classification, it has been observed (Nair, 1970; see also Erdtman, 1952) that the Ranales of Bessey if taken to be the most primitive order, consists of two definite palynological stocks, namely the "Magnolian stock" and the "Ranalian stock", represented respectively by Magnoliaceae with monocolpate pollen, and Ranunculaceae with fundamentally tricolpate pollen. Further, the occurrence of new aperture morphoforms, represented by the 3-colpates and its derivatives in the "Ranalian dicots" is taken as proof to propose that the "Ranalian dicots" are the latest to evolve in the phylogenetic history of the plant kingdom (see Fig. 1). It is in this background that a comparative examination of the various current systems of classification of angiosperms with reference to pollen morphology, is presently attempted.

CURRENT SYSTEMS OF ANGIOSPERM CLASSIFICATION

Among the current systems of angiosperm classification, those of Bentham and Hooker, Engler (see Willis, 1966), and Hutchinson (1926) are the best known, and more universally followed. Further, the system of Cronquist (1968) project some modern ideas on the subject, meaning those of Takhtajan. However, the system of Hallier, Bessey, Tippo, Pulle, Skottsberg, Rendle and Wettstein do bear considerable significance in comparative studies of taxonomic and phylogenetic relationships of angiosperm taxa (see Lawrence, 1951).

The system of classification of angiosperms by Bentham and Hooker, is known to be artificial and suited for making a convenient herbarium categorisation of plants. All the same, the system does have the semblence of a natural classification, projecting the taxonomic affinities of plants, to a considerable degree. The main reason for calling it an artificial system of classification has been the formation of the group Monochlamydeae, but for which the classification agrees with many of the phylogenetic systems, with regard to the composition and arrangement of taxa at higher or lower levels. This is an instance to corroborate the argument by Sprague (1940) that "if those parts of the natural classification of angiosperms which have been well tested can be assumed to be phylogenetic, then there is a high

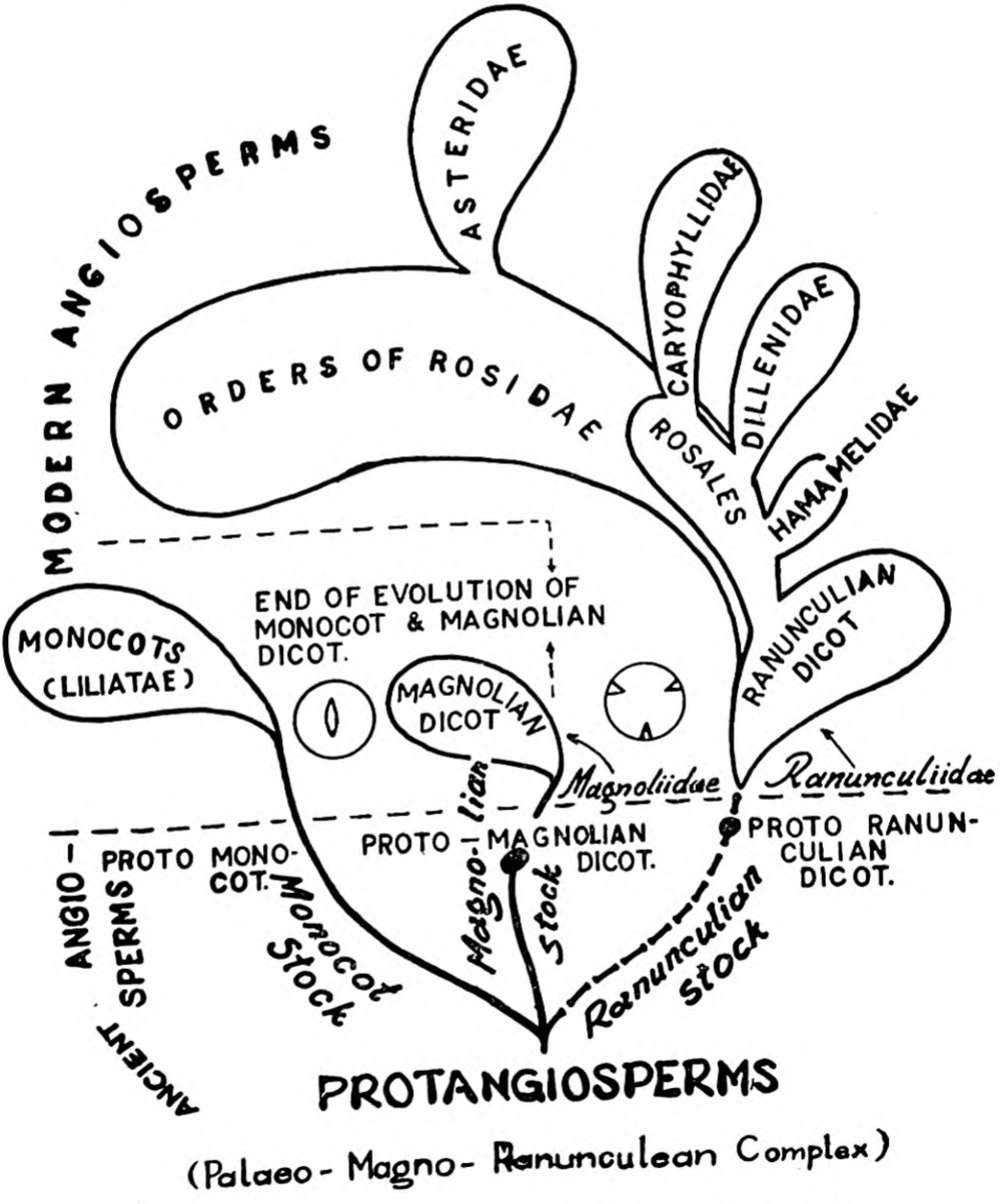

MODERN ANGIOSPERMS
ASTERIDAE
CARYOPHYLLIDAE
DILLENIDAE
ORDERS OF ROSIDAE
ROSALES
HAMAMELIDAE
RANUNCULIAN DICOT
END OF EVOLUTION OF MONOCOT & MAGNOLIAN DICOT.
MONOCOTS (LILIATAE)
MAGNOLIAN DICOT
Magnoliidae
Ranunculiidae
PROTO MONO-COT.
PROTO-MAGNOLIAN DICOT.
PROTO RANUNCULIAN DICOT.
ANGIOSPERMS
ANCIENT
Monocot Stock
Magnolian Stock
Ranunculian Stock
PROTANGIOSPERMS
(Palaeo-Magno-Ranunculean Complex)

FIG. 1. Phylogenetic position of angiosperm taxa (Modified from Cronquist, 1968).

degree of probability that certain biological progressions have taken place independently along several, even many lines of descent" (see Wardlaw, 1952, p. 104).

Bentham and Hooker divided the angiosperms broadly into the Dicotyledonae and Monocotyledonae, a classification accepted by all systematists for over a century since John Ray proposed such a division. The division of dicots into Polypetaleae, Gamopetaleae and Monochlamydeae (Apetalae) and the further categorisation of each is regarded as artificial and the arrangement of the various taxa is not necessarily an index of their relationships. However, the phylogenetically primitive taxa are placed in the Ranales, which form the first order of dicots.

The Engler's system of classification, though originally intended to have been one based on form relationships, has come to be interpreted and understood as a phylogenetic classification. The sequence of the various taxa does connote a phylogenetic meaning, and the groupings indicate the progression of evolutionary lines from apetaly to polypetaly and gamopetaly, apocarpy to syncarpy, hypogyny to epigyny, and actinomorphy to zygomorphy, and conforming with the principle of polyphyletism in evolution. Further, the monocots precede the dicots and the latter is divided into Archechlamydeae (Polypetalae and Monochlamydeae together of Bentham and Hooker) and Sympetalae. The 'monochlamydous' taxa (i.e., those with reduced floral members), precede the polypetalous and gamopetalous taxa, based on the view that the primitive flower has been of the simple (reduced) type. Most taxa of the Monochlamydeae of Bentham and Hooker are among the first seventeen orders in Engler's system, and this is followed by the Ranales and a few other thalamiflorous taxa. The composition of many orders in both systems is the same, although not as a rule.

Bessey proposed a phylogenetic classification dividing the angiosperms into two classes Alterniflorae (monocots), and Oppositifoliae (dicots) and each of the above are again divided into two subclasses namely the Strobiloideae and the Cotyloideae. He subscribed to the strobiloid theory of the origin of the flower and to the polyphyletic theory of the origin of seed at higher levels. The first order of the dicots is the Ranales which consists essentially of the families of Ranales, Piperales, and Leitneriales of Engler. He suggested the origin of monocots from a section of the dicots.

Hallier also proposed the origin of monocots from dicots and he divided the Ranales of Bessey into "Ranales" and "Annonales", the former represented by the Ranunculaceae and the latter by the Magnoliaceae. He also subscribed to the polyphyletic theory of angiosperm evolution.

Hutchinson also regarded the monocots as having originated from dicots (Herbaceae) which in turn originated from the hypothetical proangiosperms and diverged early into two lines, the Herbaceae and the Lignosae. The primitive families of Herbaceae and Lignosae are the Ranales and Magnoliales respectively which together form a part of the Ranales of Bessey.

Cronquist (1968), following Takhtajan, divided the angiosperms into the classes Magnoliatae (dicots) and Liliatae (monocots), the former being subdivided into six subclasses (Magnoliidae, Hamamelidae, Caryophyllidae, Dillenidae, Rosidae, and Asteridae), and the latter into four subclasses (Alismatidae, Commelinidae, Arecidae, and Liliidae). It may be noted that there is no categorisation of dicot orders into Polypetalae and Gamopetalae, although the subclass Asteridae in effect contains gamopetalous orders alone. The system has its own merits and appears more near to phylogenetic facts and natural relationships in many instances.

CLASSIFICATION OF PRIMITIVE TAXA OF DICOTS

Basal taxa.—Considering the various systems of classifications the Ranales of Bessey (as compared with the Ranales of other systematists) may be taken to form a phylogenetic base, because it contains nearly all families of primitive plants, considered from the point of view of pollen morphology (Nair, 1970). Moreover, considering all the earlier phylogenetic systems (Bentham and Hooker, Hallier, Hutchinson and Bessey) together, a large majority of the families of primitive dicots are pooled together in the Ranales of Bessey, except for Nymphaeaceae, which is placed in the Rhoedales, and Aristolochiaceae placed in the Myrtales. By adopting the Ranales of Bessey added with the families Nymphaeaceae and Aristolochiaceae, for the sole purpose of initiating the present discussion, the author does not mean to discount the categorisation of the primitive dicots into more than one order, synthesized in a primitive dicots-complex such as the Magnoliidae of Cronquist (1968).

As already indicated, the Ranales of Bessey consists of two distinct "palynological stocks", namely the monocolpate (dominantly monocolpate), and the tricolpate (dominantly tricolpate) stocks, represented by the Magnoliaceae (i.e. Magnolian stock), and the Ranunculaceae (i.e. Ranalian stock, better say Ranunculian stock) respectively. The "Magnolian" stock contains the families Magnoliaceae, Calycanthaceae, Monimiaceae, Saururaceae, Piperaceae, Chloranthaceae, Lauraceae, Lacistemaceae, Cobombaceae, Ceratophyllaceae, and Winteraceae. The "Ranalian" stock is constituted of Ranunculaceae, Lardizabalaceae, Berberidaceae, Menispermaceae, Nelumbaceae and Dilleniaceae.

A comparative study of the classification of "Ranales" by the various taxonomists suggests that the above families constituting the "Magnolian" stock and the "Ranalian" stock essentially belong to two orders namely the Magnoliales and Ranales (in the restricted sense) respectively, each subdivided sometimes into a greater number of orders (see Hutchinson, 1969; Cronquist, 1968). Hutchinson restricted the taxonomic limits of the Ranales to the Nymphaeaceae, Ranunculaceae, and Berberidaceae, of which Nymphaeaceae possesses 1-colpate pollen (barring Nelumbonaceae), and the others have the basic 3-colpate forms. All other monocolpate families are distributed in Magnoliales, Laurales, and Piperales.

In Engler's system, a majority of the monocolpate taxa of dicots are accomodated in the Ranales, but for Piperales and Winteranaceae (Canellaceae), which are placed in Piperales and Parietales respectively. Hallier proposed three orders, namely Ranales (containing taxa with 3-colpate families, but for Nymphaeaceae), Annonales and Piperineae (monocolpates). Cronquist (1968) distributed the monoaperaturate taxa in the Magnoliales, Piperales, Aristolochiales and Nymphaeales, which along with

Ranunculales and Papaverales having the fundamental 3-colpate pollen, form the subclass Magnoliidae. In the system of Bessey alone, almost all the monocolpate taxa of dicots find a place within the Ranales (including Nymphaeaceae and Aristolochiaceae).

On the basis of palynological data, it has been argued (Nair, 1970) that the trichotomocolpates, monocolpates and their derivative inaperturates, must be the most primitive pollen morphoforms, because they reflect the trend of morphological evolution that was organised in the bryophytes primarily, and repeated in the pteridophytes and the gymnosperms. Hence, a classification that accomodates almost all the monocolpate dicots within its basic orders should be favoured in terms of pollen phylogeny, and in this regard, the systems of Bessey, and Cronquist obtain preference over others in regard to the primitive taxa of angiosperms.

As indicated earlier, the Ranales of Bessey contain two clear palynological stocks, represented by the fundamentally monocolpate sporomorphs and the fundamentally tricolpate sporomorphs, designated as the "magnolian" stock and the "ranalian" stock respectively. The subclass Magnoliidae of Cronquist also accommodates all the monocolpate dicots within it, but circumscribed into four orders namely, Magnoliales, Piperales, Aristolochiales and Nymphaeales and in the light of this the comparative merit of the system of Cronquist over that of Bessey is discussed below:

Compared with the system of Bessey, the system of Cronquist finds greater acceptance for the simple fact that all the monocolpates (including Aristolochiaceae and Nymphaeaceae), which is undoubtedly a symbol of primitiveness, are brought under a common subclass Magnoliidae although the monocolpate taxa are further divided into four orders namely, Magnoliales, Piperales, Aristolochiales, and Nymphaeales (barring the 3-colpate Nelumbonaceae). However, Cronquist does not include in his Ranunculales all the tricolpate families assigned to Ranales by other systematists, but includes the controversial family Coriariaceae, and also Sabiaceae in it.

From palynological evidences it is suggested that the orders of the subclass Magnoliidae of Cronquist should be divided into two sub-classes namely, the Magnoliidae representing the monocolpate magnolian stock of dicots composed of the orders Magnoliales, Piperales, Aristolochiales and Nymphaeales (barring Nelumbonaceae), and the Ranunculiidae comprised of the orders Ranunculales (with the addition of Nelumbonaceae) and Papaverales. The Magnoliidae and Ranunculiidae together should be considered to form the Magno-Ranalian complex (i.e., a complex of present day primitive taxa reflecting the hypothetical protangiosperms of which there is no clear fossil data; see Fig. 1), equivalent to the Ranales alone of Bessey (including Nymphaeaceae and Aristolochiaceae).

Evolution of other dicots taxa.—Considering that the Magnoliidae of Cronquist (equivalent to the Ranales of Bessey with the addition of Nymphaeaceae and Aristolochiaeceae; i.e. Magno-Ranalian complex) represents the primitive stage of evolution of the dicots, it is apparent that the Magnolian palynological stock (i.e., Magnoliidae) did not proceed beyond the basal primitive level. Consequently, all other orders of dicots are phylogenetically related to the 3-colpate Ranunculian palynological stock (i.e., Ranunculiidae).

In the various phylogenetic schemes of classification, the orders evolving from the Ranales sensu lato (i.e. Ranalian and Magnolian families together), are shown to be either monophyletic or polyphyletic. Bessey (see Lawrence, 1951), regarded the Ranales to have given rise to the Rosales, which in turn gave rise to various orders of dicots polyphyletically. Hallier believed in a polyphyletic origin of Nepenthales, Caryophyllinae, Guttales, Proteinae, Columniferae, and Annonales (sensu stricto, in comparison to which Bessey derived all the above orders from the Rosales. Hutchinson derived all the dicot orders along two lines, the Lignosae, and the Herbaceae, from the basal taxa Magnoliales (sensu stricto) and the Ranales (sensu stricto) respectively, which is apparently not in line with palynological facts. Cronquist (1968) derived the Hammamelidae, Dillenidae, Caryophyllidae, and Rosidae, directly from the Magnoliidae, while the Asteridae has been derived from the Rosidae.

By being dominantly polycarpous, 3-colpate and multipalynous the order Rosales is phylogenetically related to the Ranales, to a greater degree than other orders, and Bessey considered it to be the connecting link between the Ranales on the one hand and the higher orders on the other. Do Rosales represent a second stage in the evolution of the primitive dicots ? In this connection it is of interest to note that Cronquist also considered the Rosales to be the only phylogenetic base for the Asteridae (i.e., Sympetalae) while all other subclasses of dicots (Magnoliidae) are shown to have evolved independently of each other. Further, it may be presumed that the origin of new taxa may be sudden or slow, depending upon the nature of the genetical process. Every new major origin may also be a monophyletic phenomenon, and completed in one or more steps. This is succeeded by the processes of natural selection leading to the channelisation of desired variations along different lines of stability (polyphyletic) resulting in the ultimate establishment of stable taxa. This process is reflected in pollen by the decreasing extent of multipalyny leading to the final establishment of unipalyny.

The polycarpic and multipalynous Ranales and Rosales may represent two succeeding steps in the establishment of the new origint (i.e. angiosperm) in the evolution angiospersm of the plant kingdom. It is therefore felt that there has been a two-tier evolution of the primitive taxa of dicots followed by the polyphyletic origin of the higher taxa. In such a scheme, the Hamamelidae, Caryophyllidae, and the Dillenidae of Cronquist may be considered to have diverged from the lower levels and the Asteridae from the higher level of the Rosalian plexus (Fig. 1).

CLASSIFICATION OF PRIMITIVE TAXA OF MONOCOTS

The monocots are considered to be a natural group of taxa by all systematists. The group is dominantly monocolpate and although the 3-colpates occur only sporadically, pollen morphotypes which are derivatives of the 3-colpate fundamental form, are noticeably present. However, these derivatives may be considered to have their roots in the commonly occurring trichotomocolpates, and thus the monocots as a whole may be deemed to belong to the monocolpate palynological stock, and may have originated from the hypothetical protangiosperms (Nair, 1970) indepentdently of the magnolian monocolpate stock. It has been observed earlier

(Nair, 1970) that the monocots and magnolian dicots reflect in them a common trend of evolution initiated in the pre-angiosperm phyla, namely the bryophytes, pteridophytes and gymnosperms, and it is therefore logical to assume that they were the first to appear in the wake of the origin of angiosperms.

In the various systems of phylogenetic classifications, the primitive taxon is believed to be Liliiflorae by Hallier, Alismatales by Bessey, Alismaticidae by Cronquist, Butomales and Alismatales by Hutchinson and all these systematists derived the monocots from the Ranales of dicots. Engler believed that the monocots preceded dicots in origin.

The monocots, by their characteristic morphology in all aspects of structural organisation, having very little in common with the trends of evolution in dicots, should be considered to have originated independently of dicots, the confluence of the dicots and monocots at most having occurred in the hypothetical protangiosperms. The occurrence of monocolpates in the 'magnolian' dicots and monocots, of the present day plants is perhaps only a reflection of homoplasy in evolution.

The primitive taxon may be one in which a complex of morphoforms, occur in a manner similar to that in the magnoranalian complex, and this principle is exemplified in the Liliiflorae of Hallier, comprised of the Liliaceae and the Amaryllidaceae. On many other morphological considerations, the Alismatales has been considered by a majority of modern systematists to be the primitive taxon of the monocots. However, Hallier considered the Helobiae (containing also the Alismaceae) and every other order of the monocots to have derived from the Liliiflorae, independent of each other. Moreover, the occurrence of the advanced pantoporate pollen type in Alismataceae negatives the belief regarding the primitiveness of Alismatales.

Compared with the dicots, the palynological evolution of the monocots was only the repetition of an already established trend of morphological evolution of the pre-angiosperm phyla. Hence the establishment of the evolutionary trend in the monocots might be considered to have been accomplished in step, at the level of the Liliiflorae itself. Further evolution might have been essentially polyphyletic as suggested by Hallier (see Lawrence, 1951). However, the taxonomic circumscription of every order requires a critical re-assessment in the light of the suggestion given by the author (Nair, 1970) for the reclassification of monocots.

REFERENCES

AXELROD, D. I. 1970. Mesozoic phytogeography and early angiosperm history. *Bot. Rev.* **36**: 277-319.

BOCK, W. J. 1970. Microevolutionary sequences as a fundamental concept in macroevolutionary models. *Evolution*, **24**: 704-722.

BRIGGS, D., AND S. M. WALTERS. 1969. *Plant Variation and Evolution*. World University Library, London.

CRONQUIST, A. 1968. *The Evolution and Classification of Flowering Plants*. Thomas Nelson and Sons Ltd. London.

CROWSON, R. A. 1970. *Classification and Biology*. Heinemann Educational Books. London.

CUTTER, E. G. 1966. *Trends in Plant Morphogenesis*. Longmans, London.

DUDDINGTON, C. L. 1969. *Evolution in Plant Design*. Faber and Faber Ltd., London.

ERDTMAN, G. 1952. *Pollen Morphology and Plant Taxonomy/Angiosperms*. Almqvist and Wiksells, Stockholm and New York.

——. 1957. *Pollen and Spore Morphology/Plant Taxonomy. Gymnospermae. Pteridophyta and Bryophyta*. Almqvist and Wiksell Stockholm.

Erdtman, G. 1965. *Pollen and Spore Morphology/ Plant Taxonomy. Gymnospermae, Bryophyta (Text)*. Almqvist and Wiksell, Stockholm.

Good, R. 1956. *Features of Evolution in the Flowering Plants*. Longmans, Green and Co., Lodnon.

Gunckel, J. E. (ed.). 1969. *Current Topics in Plant Science*. Academic Press, New Yrok.

Harborne, J. B. (ed.). 1970. *Phytochemical Phylogeny*. Academic Press, London and New York.

Hutchinson, J. 1926. *The Families of Flowering Plants. Vol. I. Dicotyledons*. Oxford. Clarendon Press.

Hutchinson, J. 1934. *The Families of Flowering Plants. Vol. II*. Monocotyledons. Oxford. Clarendon Press.

Lawrence, G. H. M. 1951. *Taxonomy of Vascular Plants*. The MacMillan Co., New York.

Manwell, C., and C. M. Baker. 1970. *Molecular Biology and the Origin of Species*. Sidgwick and Jackson, London.

Margoliash, E., and W. M. Fitch. 1971. Amino-acid sequences and phylogeny. *Taxon*. **20**: 51-53.

Mayr, E. 1963. *Populations, Species and Evolution*. The Belknap Press of Harvard University Press, Cambridge, Massachusetts.

Mehra, P. N. 1968. Palynology of the Archegoniatae—An Evolutionary Approach. *J. Palynol*. **4**: 56-72.

Nair, P. K. K. 1970. *Pollen Morphology of Angiosperms—A Historical and Phylogenetic Study*. Vikas Publications, Delhi/Scholar Publishing House, Lucknow.

Sprague, T. A. 1940. Taxonomic botany with special reference to the angiosperms. In *New Systematics*. Turill (ed.) Oxford University Press, London.

Stebbins, G. L. 1971. Relationships between adaptive radiation speciation and major evolutionary trends. *Taxon* **20**: 3-16.

Wardlaw, C. W. 1952. *Phylogeny and Morphogenesis*. Macmillan and Co., London.

Wardlaw, C. W. 1968. *Essay on Form in Plants*. Manchester University Press, New York.

Willis, J. C. 1966. *A Dictionary of Flowering Plants and Ferns* (Revised by Airy Shaw). Cambridge University Press.

Young, D. J., and L. Watson, 1970. The classification of dicotyledons. A study of upper levels of the heirarchy. *Aust. J. Bot.* **18**: 387-426.

Adv. Pl. Morph. 1972: 264–277

FLORAL MORPHOLOGY AND EMBRYOLOGY OF *MYRISTICA MALABARICA* LAMK. WITH A DISCUSSION ON CERTAIN ASPECTS OF THE SYSTEMATICS OF MYRISTICACEAE[1]

By N. C. Nair

Botanical Survey of India, 76, Acharya Jagdish Bose Road, Calcutta-14 India

ABSTRACT

Floral morphology and embryology of *Myristica malabarica* are compared with those of *M. fragrans*. The anther wall consists of 5 layers of cells of which the innermost layer develops into a secretory tapetum whose cells remain uninucleate throughout. The subepidermal layer develops into a fibrous endothecium. Microspore mother cells are in a single row. The microsporangium is divided into two or three compartments by sterile septa. The tetrads are isobilateral. Pollen grains have reticulate exine and are shed at the 2-celled stage. The ovule is anatropous and bitegmic to begin with. At the mature embryo sac stage the primordium of a middle integument develops at the chalazal end. It remains arrested and gets crushed during the development of seed. The nucellus has a prominent cap. Embryo sac is of the 8-nucleate Polygonum type. Pollen tube destroys one of the synergids in effecting fertilization. Endosperm is Nuclear. Massive chalazal growth takes place after fertilization which pushes the inner integument and nucellus upwards. Rumination in the endosperm is brought about by ingrowths from the inner seed coat. The aril develops from the funicle as well as from the tip of the outer seed coat. The inner epidermis of the outer seed coat forms the mechanical tissue in the seed. Both the seed coats are vascularized. The embryo development appears to be of the Onagrad type but this needs confirmation. Interelationships of the Myristicaceae are discussed and it is proposed that the family be kept under a separate order Myristicales.

INTRODUCTION

The Myristicaceae is a purely tropical family of doubtful systematic position. Baillon (1872), taking into account the flower and seed allied it with the Lauraceae, Proteaceae, Monimiaceae, Menispermaceae and Lardizabelaceae. Bentham and Hooker (1883), placed it along with Piperaceae, Chloranthaceae and Monimiaceae. Warming and Potter (1932) treat it in the Polycarpicae after Lauraceae. Hallier (1905, 1912), Bessey (1915) and Engler and Gilg (1924) include it in the Ranales along with Menispermaceae, Magnoliaceae, Annonaceae and Lauraceae. Hutchinson (1926, 1959) separated it to Laurales and considered (1969) it to be a climax family of the order. Garret (1933) also, taking into account the anatomical features of the wood, linked it with Lauraceae a view which was also held by Smith and Wodehouse (1938).

---

[1] Most part of this work was carried out at the Birla College of Science (now Birla Institute of Technology and Science), Pilani.

Joshi (1946), on the otherhand, finds a close relationship between Myristicaceae and Annonaceae as suggested by Wettstein (1925). This view has been supported by Erdtman (1952) on palynological grounds. According to Sporne (1949) the family occupies the third primitive-most position among dicotyledons. Corner (1949 a, 1954) regards that it is a very primitive and peculiarly isolated family being the only entirely arillate family among the angiosperms. Sastry (1954) found similarities with Monimiaceae and Nymphaeaceae. Takhtajan (1966) includes Myristicaceae in Magnoliales and considers related to Canellaceae a view held by Vestal (1937), Parameswaran (1962) and Wilson and Macaulens (1967). Cronquist (1968) thinks that it is allied to the Magnoliaceous group of families. Sinclair (1958) regards the family as relict one, of the early Jurassic or Cretaceous (Croizat, 1952), and is related to the Annonaceae than to any other family. Inspite of the uncertain systematic position very few attempts have been made to understand the floral morphology and embryology of this puzzlanimous family of flowering plants.

PREVIOUS WORK

Voigt (1888) made some observations on the phenomenon of rumination in the endosperm and seed coat structure of *Myristica fragrans*. Joshi (1943) discussed the origin of trimerous perianth and (1946) studied the development of pollen grains in the same species. Sastry (1954, 1959) studied the anatomy of the female flower of *M. fragrans* and gave (1955) an account of the development of the seed and aril. Periasamy (1961) discussed the morphology of ruminating tissue and seed coat of *M. fragrans* and corrected some of the earlier mistakes. Studies on the floral morphology of *M. malabarica* were made by Nair and Bahl (1956) and Pillai (1959) and of *M. fragrans* by Nair and Pillai (1959). Floral morphology of these two species were also studied by Wilson and Macaulens (1967). Smith and Wodehouse (1938) gave an account of the pollen grains of some American species of Myristicaceae. The pollen morphology of three Indian species of the family was given by P. K. K. Nair (1965). Camp and Hubbard (1963) observed that the vascularization of the aril in *M. fragrans* has considerable similarity with the branched structure from which the cupule of Lower Carboniferous Pteridospermae has been derived. Bhandari (1971) while dealing with the embryology of Magnoliales made reference to the features of Myristicaceae.

MATERIALS AND METHODS

The present work mainly deals with *M. malabarica*. For comparison *M. fragrans* Van Houtten was also studied. Both the species are of considerable economic importance and are, therefore, often found in cultivation. The materials were fixed in formalin-acetic-alcohol (FAA) and later transferred to 70 per cent alcohol. Materials of *M. malabarica* were obtained from plants growing in Rajan's Estate, Thankamani, Kerala, and that of *M. fragrans* from plants cutivated in the neighbourhood of Kottayam, Kerala. Paraffin embedded flowers and young fruits were cut at a thickness of 8-12 microns. Older seeds were treated with 5 per cent hydroflouric acid in 70 per cent alcohol for four weeks before embedding and were cut at 16 microns thickness. Mature seeds were sectioned on a Jung's sliding microtome at a thickness of 18 microns. Staining was done with iron-alum haematoxylin as well as safranin-fast green. Aril was cleared with 5 per cent chloral hydrate in water at 60°C for 24 hours and later stained with aquous basic-fuchsin.

OBSERVATIONS

Microsporangium and microsporogenesis.—The stamens are united to form a solid column the lower portion of which is differentiated into a short stalk while the upper bears 25-30 anthers in *M. malabarica* and 15-20 anthers in *M. fragrans.* The anthers are extrorse having two locules each. The connectives are fused to form a cap-like structure at the tip of the column.

A row of hypodermal archesporial cells gets differentiated in each microsporangium. At a later stage there are five wall layers surrounding the microspore mother cells (Fig. 1). Of these the innermost develops into a secretory tapetum whose cells remain uninucleate (Fig. 4) throughout as has been reported by Joshi (1946). The tapetal cells disintegrate very late and they completely disappear only when the anther is nearing dehiscence. The subepidermal layer develops into an endothecium whose cells show characteristic fibrous thickenings (Fig. 9) only after 2-celled pollen grains are distinguished in the microsporangium. The middle layers disappear before the fibrous thickenings are visible in the endothecium.

The microspore mother cells are arranged in a single row in both the species. One significant feature of the microsporangium is the presence of sterile septa which divide the sporangium into two or three compartments (Figs. 4-7). Origin of the sterile septa could not be studied. The septa are 3-5-celled in thickness and the cells lying in contact with the developing pollen grains are tapetal in nature (Fig. 7). The microspore tetrads are isobilateral (Fig. 2). The pollen grains do not round off immediately after their liberation from the mother cells and continue to occupy for some time the same position they had at the tetrad stage (*see also* Joshi, 1946).

Male gametophyte.—The young microspore has a prominent nucleus surrounded by dense cytoplasm. As it increases in size, its nucleus is shifted to one side by the formation of a large vacuole in the centre (Fig. 7). Its wall gets differentiated into a thick exine and a thin intine.

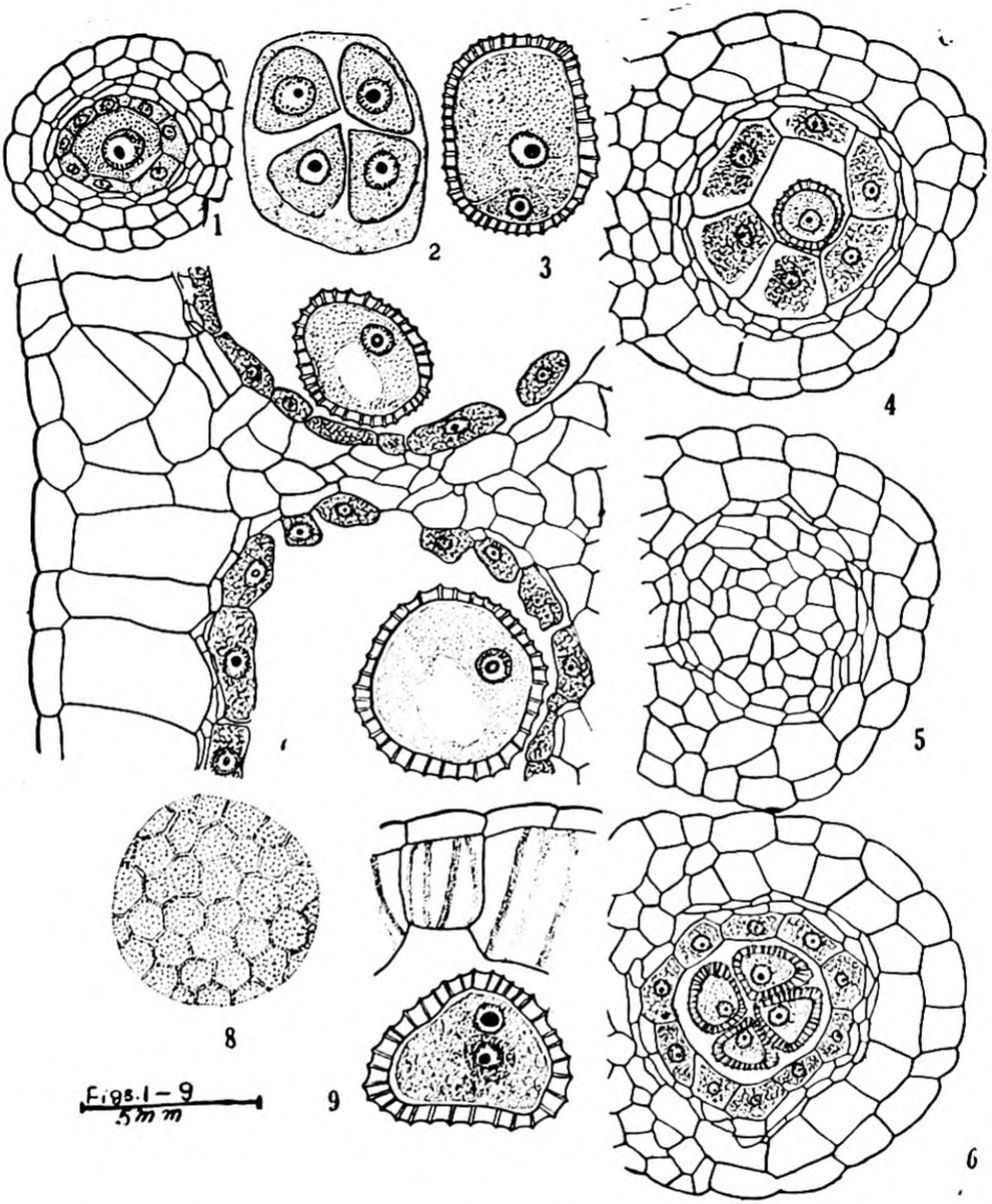
The division of microspore nucleus results in the formation of a large vegetative cell and a small generative cell. They are separated by a hyaline space (Fig. 3). The generative cell is later embedded in the cytoplasm of the vegetative cell (Fig.9).

The pollen grains are shed at the 2-celled stage. The mature grains* are circular in outline, 45-51μ. Sexine thicker than naxine; reticulate, heterobrochate, 1-colpoid, furrow 23.4 x 11.7μ.

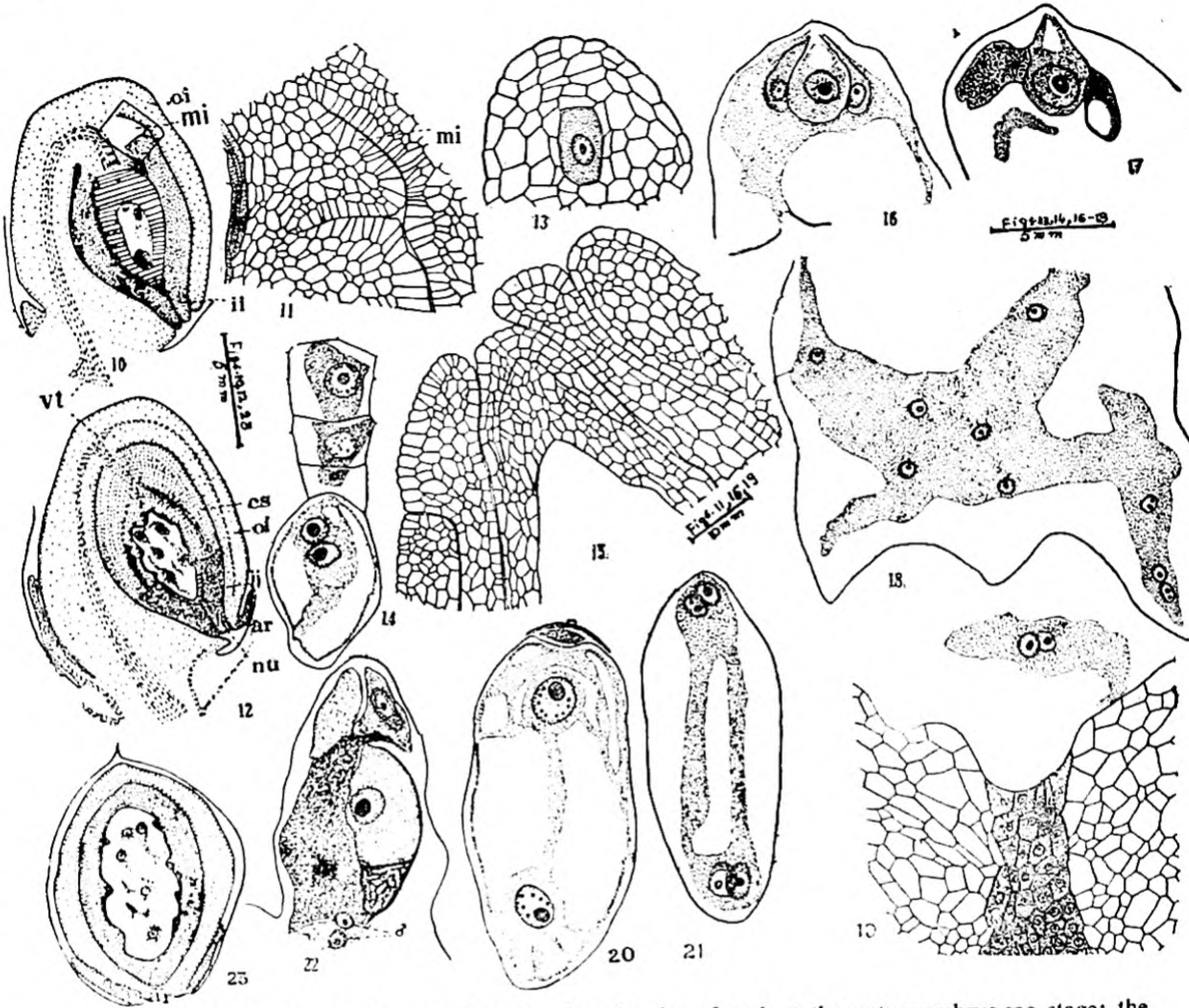
Megasporangium and megasporogenesis.—The sub-basal ovule is bitegmic to start with. It is anatropous and crassinucellate. The inner integument takes part in the formation of the micropyle (Figs. 10, 15). Both the integuments are massive at the tip. The outer integument is free from the inner up to the chalazal end (Fig. 10). The inner integument is free from the outer as well as from the nucellus up to the chalaza (Fig. 10). This distinction is very clear in young ovules. The inner integument and the nucellus are pushed up during postfertilization stages due to meristematic activity of the chalazal zone (Fig. 12) and as a

---

* I am thankful to Mr. N. G. Lukose, Palynologist, Oil and Natural gas Commission, Jodhpur, for providing the description of the pollen grain.



Figs. 1-9. *M. malabarica*. Fig. 1. Transection of anther lobe showing microspore mother cell surrounded by five wall layers. Fig. 2. Microspore tetrad. Fig. 3. 2-celled pollen grain, the generative cell is separated from the vegetative cytoplasm by a hyaline membrane. Figs. 4-6. Three consecutive transections of the anther. Fig. 7. Longisection of anther showing sterile septum and pollen chamber surrounded by tapetal cells and wall layers. Fig. 8. Surface view of mature pollen. Fig. 9. Transection of portion of mature anther showing epidermis fibrous endothecium and 2-celled pollen grain.



FIGS. 10-23. *M. malabarica* Fig. 10. Longisection of ovule at the mature embryo sac stage; the primordium of aril is seen at the base of the funiculus (left). Fig. 11. Enlarged view of portion marked by a square in the chalazal region of fig. 10, showing the primordium of middle integument. Fig. 12. Longisection of the developing seed at the nuclear stage of the endosperm; the inner integument and nucellus are pushed up by perichalazal growth. The chalazal vascular strand extends up to the base of the inner integument and almost to the tip of the outer integument; aril arises from the tip of the outer integument as well as the base of the funicle; aril arising from the funicle receive independent vascular strand. Fig. 13. Longisection of nucellus showing megaspore mother cell and 5 layers of parietal layers. Fig. 14. Linear tetrad of megaspores. Fig. 15. Micropylar region of the ovule at the mature embryo sac stage. Fig. 16. Egg apparatus. Fig. 17. Degenerating egg apparatus. Fig. 18. Chalazal region of the embryo sac showing free nuclear endosperm. Fig. 19. Chalazal region of the ovule showing dark staining cells between vascular strand and embryo sac. Figs. 20, 21.2-and 4-nucleate embryo sacs. Fig. 22. Fertilization; the large pollen tube has destroyed one of the synergids; the sperms have very prominent nuclei. Fig. 23. Longisection of the developing seed at nuclear stage of the endosperm; note the intrusions by the inner seed coat and the aril arising from the base of the funiculus. new; *ar*, aril; *cs*, chalazal tissue formed by chalazal growth; *ii*, inner integument; *is*, inner seed coat; *mi*, middle integument; *nu*, nucellus; *oi*, outer integument; *vt*, vascular tissue.

result of which a false impression may be created that the inner integument arises from the upper portion of the nucellus. The epidermis of the outer integument contains a few stomata in both the taxa (*see also* Sastry, 1955). The outer and inner integuments are 5-7-layered and 4-5-layered in thickness respectively at the mature embryo sac stage (Fig. 29). The vascular supply to the ovule terminates at the chalaza (Fig. 10). Between the vascular strand and the embryo sac can be seen 5-6 rows of differently staining cells (Fig. 19).

An unusual feature of both the species is the development of the primordium of a middle integument between the inner and the outer integuments at the mature embryo sac stage (Figs. 10, 11). The middle integument arises only on the antiraphe side in the form of a collar and it never becomes a sheathing structure. Development of the middle integument is arrested at a very early stage and therefore it does not contribute to the formation of the seed coat.

The nucellus is massive. The nucellar epidermis undergoes periclinal divisions with the result a cap of two or three layers is formed (Fig. 15). At the time of fertilization there are seven to eight layers of nucellar cells in the micropylar region and four to six layers on the sides of the embryo sac.

The youngest ovule of *M. malabarica* in my possession showed a megaspore mother cell with 4 parietal layers (Fig. 13). In *M. fragrans* also the megaspore mother cell was deep seated. Magaspore tetrads are linear (Fig. 14). The chalazal megaspore alone develops further while the others degenerate (Figs. 14, 20).

Female gametophyte.—The functional magaspore nucleus divides and the daughter nuclei occupy opposite poles (Fig. 20). They undergo two more divisions resulting in an eight nucleate embryo sac (Figs. 10, 16, 21, 24, 26) of the Polygonum type (Maheshwari, 1950), as reported by Sastry (1955). The egg apparatus has the usual organization (Fig. 16). In most cases the synergids were taking a very deep stain and show signs of degeneration (Fig. 17). The egg nucleus is very prominent when compared to the synergid nuclei (Fig. 16). The polar nuclei fuse at the chalazal end of the embryo sac (Figs. 24, 26). The antipodals are triangular and persist till about the time of fertilization. The mature embryo sac is broader near the chalazal end.

Fertilization.—*Myristica* is entomophilous. The entry of pollen tube is porogamous as has been reported by Sastry (1955). Sastry reports that the pollen tube persists till a young embryo is formed. This is not the case with my material. The pollen tubes are ephemeral and no trace of them could be observed in both the species after about 16 endosperm nuclei are formed. The pollen tube destroys one of the synergids while entering the embryo sac (Fig. 22). The tip of the pollen tube is very broad. When discharged from the tube, the nuclei of the sperms are very distinct and have prominent nucleoli.

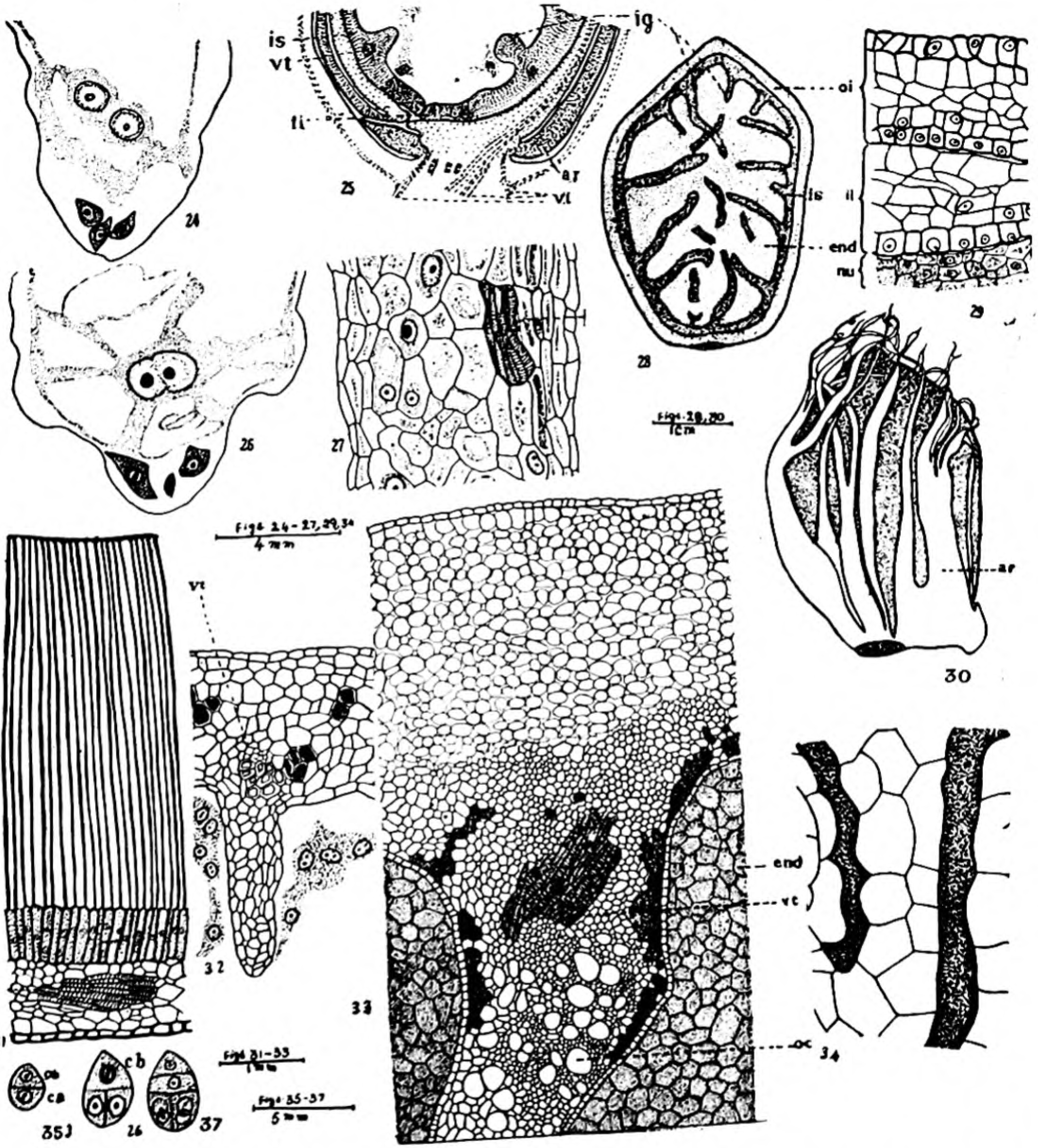
Chalazal growth.—After fertilization, the outer integument as well as the tissue that lie inside it and immediately below the region from where the inner integument arises become very actively meristematic and grow rapidly. Neither the inner integument nor the nucellus show such an activity. Not only that, they remain as they were before fertilization. As a result of the activity of the chalazal meristem the inner integument is pushed towards the apex (Fig. 12) where it can be clearly marked out in the maturing seed (Fig. 25). The primordium of the middle integument is crushed out of shape in the enlarging seed. The vascular strand at the chalazal region undergoes extensive ramification in the inner marginal region of the tissue produced by chalazal hypertrophy. The vascular strand also extends into the tissues newly formed in the outer integument (Fig. 12) but it does not reach the tip in both the species.

Endosperm.—The primary endosperm nucleus divides earlier than the egg. Since the early divisions are not followed by wall formation the development of the endosperm is of the Nuclear type (Figs. 12, 18, 23, 32). The endosperm grows at the expense of the nucellus at first and then grows downward into the central part of the tissue produced by the chalazal activity (Fig. 12). From the region where the vascular strand lies in the chalazal tissue which surrounds the endosperm finger-like ingrowths are produced by active division of cells (Figs. 12, 23, 32). These ingrowths push the endosperm deeper and deeper as a result of which the surface of the endosperm becomes irregular (Fig. 28). Each ingrowth receives a vascular strand. The peripheral cells of the ingrowth are frequently tanniniferous (Fig. 33). There are several oil cavities in the finger-like ingrowths (Fig. 33). After several free nuclear divisions the endosperm becomes cellular. Cell formation begins from the periphery. Even after the entire endosperm becomes cellular the ingrowths remain intact and make the endosperm ruminate (Figs. 28, 33). Both the outer and inner integuments do not take part in the rumination process. The endosperm contains abundant fat, aleurone grains and starch grains.

Embryo.—Only a few stages in the development of embryo in *M. malabarica* were observed. The first division of the zygote is transverse and produces an apical cell *ca* and a basal cell *cb* (Fig. 35). The basal cell undergoes a transverse division and the apical cell undergoes a vertical division (Fig. 36). The terminal tier of two cells thus formed undergoes another vertical division (Fig. 37). Further divisions could not be followed with clarity. But it appears to follow the Onagrad type (this needs confirmation). The mature embryo is dicotyledonous and is extremely small when compared with the size of the endosperm and seed. The cotyledons are laciniate.

Seed coat.–The inner seed coat is derived from the chalazal tissue. Just before wall formation in the endosperm, the inner seed coat is 13-14 layers thick (Fig. 32). In the mature seed it is composed of 35-39 layers of thin walled cells some of which contain dark staining material (Fig. 33). In the peripheral region of the inner seed coat the cells are loosely arranged while in the inner region they are compact. It is in the compact region the main vascular strand of the inner seed coat is situated (Fig. 33).

The outer integument grows in thickness only when the seed is nearing maturity. Immediately after fertilization the inner epidermal cells of the outer integument is radially elongated, has dense cytoplasm and look like palisade. As the seed is about to mature, this layer undergoes rapid radial elongation (10-12 times) and its cell walls become sclerified. This layer forms the mechanical tissue of the seed (Fig. 31). The inner subepidermal layer of cells also elongate radially and may undergo a periclinal division (Fig. 31). These cells have dense cytoplasm and prominent nuclei. The outer epidermis become cuticularized thick walled cells. Beneath this there are 5-6 layers of parenchymatous cells. Thus the outer seed coat can be distinguished into (a) cuticularized thick walled epidermis, (b) parenchymatous zone of 5-6 layers of cells, (c) one or two layers of radially elongated cells with cytoplasm and (d) the mechanical tissue of the


is
vt
ig
fi
ar
vt
oi
is
ii
end
nu
24
25
26
27
28
29
Figs. 28, 30
1 cm
Figs. 24-27, 29, 34
4 mm
ar
30
vt
32
33
end
vt
oc
34
31
Figs 31-33
1 mm
Figs 35-37
5 mm
ob
ca
cb
35
36
37

FIGS. 24-37. Figs. 24-34. *M. malabarica*. Fig. 24. Chalazal region of embryo sac before polar fusion. Fig. 25. Longisection of micropylar region of developing seed. Fig. 26. Chalazal region of the embryo sac after polar fusion. Fig. 27. Part of aril in transection. Fig. 28. Longisection of fully grown seed (aril not shown). Fig. 29. Transection of part of ovule showing the two integuments and nucellus at the mature embryo sac stage. Fig. 30. Mature seed. Fig. 31. Transection of the mature outer seed coat. Fig. 32. Portion of the inner seed coat in transection showing ingrowth and free nuclear endosperm. Fig. 33. The same after endosperm has become cellular. Fig. 34. Part of the pericarp in longisection showing tannin tubes. Figs. 35-37. Stages in the development of embryo.

ar, aril; *end*, endosperm; *ii*, inner integument; *ig*, ingrowth from inner seed coat; *is*, inner seed coat; *nu*, nucellus; *oi*, outer integument; *vt*, vascular tissue.

seed composed of radially elongated thick walled cells.

Aril.—At about the mature embryo sac stage the peripheral cells of the funicle immediately below the ovule begin merismatic activity and produces an outgrowth (Figs. 10, 12, 23) which develops into an aril. The aril grows rapidly by the stimulation offered by fertilization and covers the seed even before the infoldings become very prominent in the inner seed coat. Immediately after fertilization the tip of the outer integument also proliferates and contributes to the formation of the aril (Fig. 12). The portion of the aril derived from the tip of the outer integument do not contain vascular strands. On the otherhand the aril arising from the funicle is vascularized (Figs. 12, 25). The funicle shows a prominent vascular strand surrounded by several smaller strands at the time of fertilization. It is the smaller strands which enter the aril (Fig. 25) and branch profusely. The aril is annular at the base and is laciniate above (Fig. 30). The lobes are dichotomously branched and they are twisted and folded to form a cone at the top. The mature aril is red in *M. fragrans* and reddish-white in *M. malabarica* and is composed of 10-12 layers of parenchymatous cells (Fig. 27). Anatomically the aril of both the species are similar.

Pericarp.—After fertilization the ovary enlarges considerably and its wall transforms into a fleshy parenchymatous pericarp containing several long tanniniferous tubes (Fig. 34). Similar tanniniferous tubes are also reported in the stem and according to Garret (1933) such tubes are nowhere found in the plant kingdom.

DISCUSSION

The microsporangium shows sterile septa in *M. malabarica* and *M. fragrans.* Such a feature though not known previously in Myristicaceae is reported in several families (Maheshwari, 1950). In the Ranales it is known in Annonaceae (Periasamy and Swamy, 1959; Parulekar, 1970).

Tapetal cells in the anther of angiosperms are usually not uninucleate. In *M. fragrans* and *M. malabarica* they remain uninucleate throughout. According to Cooper (1933) uninucleate tapetal cells are an unusual feature of angiosperms.

Voigt (1888) stated that the inner integument is inserted at half the height of the nucellus and does not arise from the basal part from where the outer integument starts. According to Sastry (1955), on the other hand, nucellus, inner integument, and outer integument are distinct up to the chalaza and it is only in older ovules that this distinction is lost. It has been observed in the present study that all the three regions are distinct in the younger ovules and the inner integument and nucellus are pushed up only after the chalazal growth starts after fertilization.

On the antiraphe side of the ovule the primordium of a middle integument is formed which remains dormant and perishes as the seed matures. A middle integument is a rare phenomenon in angiosperms and is not previously reported in Myristicaceae. In the Ranales it is known in *Canangium, Mezzettia, Xylopia* and *Cananga* (Corner 1949b; Periasamy and Swamy, 1961). Corner (1949b) thinks that the middle integument is a new intercalary feature of seed development formed as a result of extreme basipetal concentration of the growth of the ovule after fertilization, at the antimicropylar end. In *Myristica* the primordium of the middle integument is formed in the prefertilization stage as in *Cananga* (Periasamy and Swamy, 1961). Whether the primordium of the middle integument is a universal feature of the family should be ascertained by studying other members of the assemblage.

While in Annonaceae the middle integument is well developed it in Myristicaceae is arrested at the primordial stage itself. If the middle integument is a new feature, as Corner (1949b) thinks, in angiosperm evolution *Myristica* has not reached the evolutionary level of those Annonaceae members which show this peculiarity.

Morphology of the ruminating tissue in the endosperm is controversial. Voigt (1888) considered it to be a part of the nucellus itself and called it the basal nucellar portion opposed to the upper nucellar portion which is consumed by the developing endosperm. Netolitzky (1926), on the other hand, regarded it as belonging to the morphologically chalazal part. Sastry (1955) believed that the tissue is inner integument. Periasamy (1961) does not endorse the views of either Voigt or Sastry. He considers that the rumination ingrowths belong wholly to the chalaza. The present study supports the contention of Periasamy (1961).

Chalazal growth after fertilization belongs to three categories—normal-chalazal, peri-chalazal, and massive-chalazal (Periasamy, 1962). Both the species studied here belong to the massive-chalazal type (*see also* Periasamy, 1961).

The tissue called "Hauptpalisadenlage" by Voigt (1888) and which forms the mechanical tissue in the seed is interpreted as consisting of several layers by Sastry (1955) and Pillai (1959). As pointed out by Periasamy (1961), this tissue in *M. fragrans* and *M. malabarica* consists of only a single layer of radially elongated cells.

Youngken (1950), Trease (1957), and Wallis (1960) state that the seeds in *Myristica* are perispermous. They distinguished an inner perisperm and an outer perisperm and according to them, it is the inner perisperm which shows numerous extensive lamellae, corresponding to the furrows on the surface and penetrating into the endosperm. Obviously, they mean the inner integument by the term inner perisperm. The term perisperm should be restricted only to the nucellus persisting in the seed (*see* Maheshwari, 1950) as in some members of the Centrospermae. As no trace of the nucellus is found in the seeds of *Myristica* the terms inner perisperm and outer perisperm are misleading and should be avoided.

There are four views regarding the origin of aril in *Myristica* (Davis, 1966; Sastry, 1955; Sinclair, 1958). They are:— 1. It may be an aril proper, originating from the funiculus at or just below the hilum. 2. An arillode arising from around the micropyle. 3. Aril arising from funicle and micropyle. 4. An aril arising from the outer integument. The present study shows that an aril is initiated at the base of the ovule as an outgrowth of the funicle a little before fertilization. Vigorous growth of this structure takes place only after fertilization is over. The tip of the outer integument also proliferates to give rise to a small portion of the aril.

Sastry states (1955) that vascular tissue in the outer integument reaches up to the micropyle and thence into the aril. The present study could not confirm this. In both *M. fragrans* and *M. malabarica* the vascular tissue does not reach up to the tip of the outer integument. It is confined to the region newly formed as a result of post-fertilization development. Consequently the portion of the aril developed by the proliferation of the outer integument is non vascular.

SYSTEMATIC ASPECT

While any detailed consideration of the systematic aspect of the Myristicaceae requires the study of a large number of species from various genera, certain deductions can be made from the data already at hand.

Is the ruminate endosperm in *Myristica* a primitive feature ? Sinclair (1958) thought that it is derived. Some of the palynologically advanced American genera like *Compsoneura* and *Iryanthera* do not have ruminate endosperm and therefore one may be tempted to think that non ruminate endosperm is an advanced feature. Absence of rumination need not necessarily be a secondary character. But this can be decided only after data on all the genera of Myristicaceae are available.

As stated in the introduction suggestions have been put forward that the Myristicaceae is related to various families. The suggested relationships of Piperaceae, Proteaceae, Nymphaeaceae, Menispermaceae, Chloranthaceae, Lardizabelaceae, and Monimiaceae to Myristicaceae seem to be far-fetched.

A relationship between Myristicaceae and Annonaceae is attributed by several features. Both have the same geographical distribution and the same habitat, the same monopodial growth form with whorled branches and a distichous arrangement of the leaves which are themselves similar, simple and without stipules and with the veins anastomosing at the margins (Sinclair, 1958). Both have trilaucunar nodes, trimerous flowers, 5-layered anther wall with secretory tapetum and fibrous endothecium, single row of microspore mother cells, sterile septa in the anther, single-furrowed pollen grains which are 2-celled at the time of shedding, reticulate exine, anatropous bitegmic and crassinucellate ovule with post-fertilization chalazal growth, Polygonum type of embryo sac with ephemeral antipodals, Onagrad type of dictyledonous embryo, ruminate endosperm, fleshy aril and follicular fruit. In some members of the Annonaceae there is a middle integument which is also present in Myristicaceae although in a rudimentary state.

These similarities can be countered by an imposing array of dissimilarities. Garret (1933) on the basis of wood anatomy concluded that there is no similarity at all between the two families. Single flowers do not occur in Myristicaceae as in Annonaceae. While the flowers are bisexual in Annonaceae they are not only unisexual but dioecious in nearly all genera of Myristicaceae. The inner whorl of perianth is absent and the tepals are united in Myristicaceae. Trimery in Myristicaceae is derived from pentamery (Joshi, 1943; Sastry, 1954; Nair and Bahl, 1956; Nair and Pillai, 1959) and therefore affinity is to be sought among the pentatepalous families. The stamens are monadelphous and form a column in Myristicaceae. Their anthers have two loculi where as Annonaceae has embedded anthers having four loculi. Annonaceae has a thick torus while Myristicaceae does not have it. Anther tapetal cells remain uninucleate throughout in Myristicaceae and this is not the case in Annonaceae. Endosperm is cellular in Annonaceae as against nuclear in Myristicaceae. The fruits are single-seeded in Myristicaceae while they are not in Annonaceae. While the nucellus persists as oil cells in Annonaceae it is ephemeral in Myristicaceae. The nucellus develops enormously after fertilization in Annonaceae where as no such increase is met with in Myristicaceae. These differences clearly point out that the two families are not closely related. The similarities may be due to parallel development.

A Lauraceous affinity is suggested on the basis of habit, simple exstipulate leaves, superior single ovary having solitary anatropous ovule, 2-celled mature pollen grains, Polygonum type of embryo sac and nuclear endosperm. According to Garret (1933) the "similarities between the woods of these two families is outstanding". In contrast to these similarities the dissimilarities are significant. Palynologically the two families are different (Erdtman, 1952). Lauraceae has persisting epidermis of anther, amoeboid tapetum in many genera, micropyle formed by both the integuments and nonendospermic seeds which are not features shared by Myristicaceae. Lauraceae also does not show ingrowth from the inner seed coat, aril, and chalazal growth after fertilization. In the Lauraceae the seed coat is formed from the outer integument. Embryo in Lauraceae fills the entire seed. These dissimilarities point out that Lauraceae has very little relationship with Myristicaceae.

Tree habit, exstipulate, simple and alternate leaves, monadelphous androecium, extrorse anthers, simultaneous division of pollen mother cells, 2-celled monosulcate pollen grains, generative cell of the pollen grain cut off towards the proximal pole, longitudinal dehiscence of anther, crassinucellate and anatropous ovule, Polygonum type of embryo sac and ruminate endosperm are the characters shared by both Myristicaceae and Canellaceae (Parameswaran, 1961, 1962) According to Garret (1933) wood anatomical feature "although show certain similarities Canellaceae seem rather far removed from Myristicaceae". In Canellaceae the androecium is tubular; anther tapetum exhibits nuclear divisions followed by fusion and usbisch granules on the inner tangential walls; flowers are bisexual and tetra or pentamerous with perianth distinguished into sepals and petals; gynoecium is 2-5-carpellate and with many ovules on parietal placenta; micropyle is zig-zag formed by both the integuments and the seeds are nonarillate. These are significant characters not found in Myristicaceae and hence the two families cannot have any close affinity.

The view that Myristicaceae and Magnoliaceae are closely related can also be dismissed on the grounds that Magnoliaceae is a family of temperate regions and that it has 1. Multilacunar, multitraced nodes. 2. Often showy solitary, bisexual flowers. 3. Buds often subtended by bracteate spathaceous sheath. 4. Spirally disposed perianth of more than one whorl often distinguished into sepals and petals. 5. Free stamens with 1-7-traced filaments and embedded introrse anthers. 6. Division of anther tapetal nuclei. 7. Smooth exine. 8. Elongated and thick torus. 9. Many free carpels with more than one ovule in each. 10. Micropyle formed by both integuments. 11. *Ab initio* cellular endosperm which is non ruminate. and, 12. Non-arillate seeds.

From the foregoing it is clear that Myristicaceae is not closely related to any of the families mentioned above. The family shows several distinctive characters of its own and in view of these a new order Myristicales is proposed a detailed discussion about which shall be presented elsewhere.

REFERENCES

Baillon, N. 1872. *The, Natural History of Plants. Vol.* II. (English translation). London.

Bentham, G., and J.D. Hooker. 1883. *Genera Plantarum.* London.

Bessey, C. F. 1915. The phylogenetic taxonomy of flowering plants. *Ann. Mo. bot. Gdn.* **2**: 109-196.

Bhandari, N. N. 1971. Embryology of the Magnoliales. *J. Arnold. Arbcr.* **52**: 1-39.

Camp, W. H., and M. M. Hubbard. 1963. Vascular supply of the ovule and aril in peony and of aril in nutmeg. *Am. J. Bot.* **50**: 373-382.

Cooper, C. D. 1933. Nuclear division in the tapetal cells of certain angiosperms. *Am. J. Bot.* **20**: 358-364.

Corner, E. J. H. 1949a. The durian theory or the origin of modern tree. *Ann. Bot. Lond.* **13**: 367-414.

——. 1949b. The Annonaceous seed and its four integuments. *New Phytol.* **48**: 332-364.

——. 1954. The durian theory extended. II. The arillate fruit and the compound leaf. *Phytomorphology* **4**: 152-154.

Croizat, L. 1952. *Manual of Phytogeography.* The Hague.

Cronquist, A. 1968. *The Evolution and Classification of Flowering Plants.* New York.

Davis, G. L. 1966. *Systematic Embryology of the Angiosperms.* New York.

Engler, A., and S. Gilg, 1924. *Syllabus der Pflanzenfamilien.* Berlin.

Erdtman, G. 1952. *Pollen Morphology and Plant Taxonomy-Angiosperms.* Waltham Mass.

Garret, G. A. 1933. Bearing of wood anatomy on the relationships of the Myristicaceae. *Trop. Woods* **36**: 20-40.

Hallier, H. 1905. Provisional scheme of the natural (phylogenentic) system of flowering plants. *New Phytol.* **4**: 151-162.

——. 1912. L. Origine et la systeme phyletique des angiosperms. *Arch. neerl. Sci.* 111B **1**: 146-234.

Hutchinson, J. 1959. *Families of Flowering Plants. I-Dicotyledons.* (Second edition). London.

——. 1969. *Evolution and Phylogeny of Flowering Plants. Dicotyledons.* London.

Joshi, A. C. 1943. Origin of trimerous perianth. *Nature, Lond.* **152**: 695.

——. 1946. A note on the development of pollen of *Myristica fragrans* Van Houtten and the affinities of the family Myristicaceae. *J. Indian bot. Soc.* **25**: 129-143.

Maheshwari, P. 1950. *An Introduction to the Embryology of Angiosperms.* McGrawHill, New York.

Nair, N. C., and P. N. Bahl.1956. Vascular anatomy of the flower of *Myristica malabarica* Lamk. *Phytomorphology* **6**: 127-134.

——, and N. V. Pillai. 1959. Morphology of the flower and aril in *Myristica fragrans* Van Houtten. *Sci. Cult.* **25**: 211-223.

Nair, P. K. K. 1965 Pollen morphology of some families of monochlamydeae. *Bot. Notiser.* **118**: 281-288.

Netolitsky, F. 1926. *Anatomie der Angiospermen Samen, Handbuch der Pflanzenanatomie.* Berlin.

Parulekar, N. K. 1970. Annonaceae, in a comparative embryology of angiosperms. *Bull. Indian. natn. Sci. Acad.* **41**: 38-44.

Parameswaran, N. 1961. Ruminate endosperm in the Canellaceae. *Curr. Sci.* **30**: 344.

——. 1962. Floral morphology and embryology of the Canellaceae. *Proc. Indian Acad. Sci. B* **55**: 167-181.

Periasamy, K. 1961. Studies on the seeds with ruminate endosperm. I. Morphology or ruminating tissue in *Myristica fragrans. J. Madras Univ.* **31**: 53-58.

——. 1962. The ruminate endosperm. Development and type of rumination. In Plant Embryology—A Symposium. CSIR, New Delhi. 62-67.

——, and. B. G. L. Swamy. 1959. Studies in the Annonaceae—I. Microsporogenesis in *Cananga odorata* and *Miliusa wightiana. Phytomorphology* **9**: 251-263.

——, and ——. 1961. Studies in Annonacea-II. The development of ovule and seed in *Cananga odorata* and *Miliusa wightiana. J. Indian bot. Soc.* **40**: 206-216.

Pillai, N. V. 1959. *Some Observations on Floral Morphology and Embryology of Myristica malabarica.* M. Sc. Thesis, University of Rajasthan.

Sastry, R. L. N. 1954. On the vascular anatomy of the female flower of *Myristica fragrans. Proc. Indian Sci. Congr. Part III* (Abstracts) : 172-173.

——. 1955. Structure and development of nutmeg seed. *Curr. Sci.* **24**: 172-173.

——. 1959. Vascularization of the carpel in *Myristica fragrans. Bot. Gaz.* **121**: 91-95.

Sinclair, J. 1958. A revision of the Malasian Myristicaceae. *Gdns'. Bull., Singapore* **16**: 205-466.

Smith, A. C., and R. P. Wodehouse. 1938. American species of Myristicaceae. *Brittonia* **5**: 401-490.

Sporne, K. R. 1949. A new approach to the problem of the primitive flower. *New Phytol.* **48**: 259-276.

Takhtajan, A. 1966. *Systema et Phylogenia Magnoliophytorum.* Leningrad.

Trease, G. E. 1952. *A Text book of Pharmacognosy.* (6th edition). London.

Vestal, P. A. 1937. The significance of comparative anatomy in establishing relationships of the Hypericaceae to the Guttifereae and their allies. *Philipp. J. Sci.* **64**: 199-256.

Voigt, A. 1888. Untersuchungen über Bau und Entwicklung von Samen mit ruminiertem Endosperm aus den Familien der Palmen, Myristicaceen und Anonaceen. *Ann. Jard. Bot. Gard. Buitenz.* **7**: 150-190.

WALLIS, T. E. 1960. *Textbook of Pharmacognosy*. (4th edition). London.

WARMIG. H. E., AND M. C. POTTER. 1932. *A Handbook of Systematic Botany*. McGraw Hill, New York.

WETTSTEIN, R. 1925. *Handbuch der Systematischen Botanik*. Leipzig.

WILSON, T. K., AND L. M. MACAULANS. 1967. Floral morphology of Myristicaceae. I. *Flowers* of *Myristica fragrans* and *M. malabarica*. *Am. J. Bot.* **54:** 214-220.

YOUNGKEN, H. W. 1950. *Textbook of Pharmacognosy*. (6th edition). New York.

Adv. Pl. Morph. 1972: 278-290

ROOT APICAL ORGANIZATION IN VASCULAR PLANTS

By S. K. Pillai
Birla Institute of Technology and Science, Pilani, India

Abstract

Studies should be undertaken from as many aspects as possible to get a more or less complete picture of the apical organization of a root, like the ontogenetic phases during embryogeny, structural net using *Körper-Kappe* and histogen concepts, the cytohistological features, reactions to surgical experiments etc.

The reports on the occurrence of changes in the structural pattern during embryogenic studies, the development of the quiescent centre and its possible role in the regeneration of the root apex, the different structural types occurring in root apices of Pteridophytes, Gymnosperms and Angiosperms have been discussed and summarised.

Basing on the observations available certain phylogenetic trends in the evolution of root apical structures which have been suggested have been brought into focus.

Introduction

It is a great pleasure to me to associate myself with the publication of a volume commemorating the contributions Professor V. Puri has made to the cause of Botany, and to acknowledge the encouragement and unstinted help that I and my wife have received from him in our endeavours.

I have chosen this subject because I and my colleagues here have studied the root apical organization of vascular plants for some time now. Though efforts have been made to make this article comprehensive, I might have placed greater emphasis on the work we have done here and may have missed others. Some excellent reviews which have appeared in recent years are by Guttenberg (1960, 1961), Clowes (1961), Esau (1965), Fahn (1967) and Wardlaw (1968).

A study of the literature on root apical organization shows three different approaches to the problem which merge to form a connected story. They are the analysis and classification of the cell net at the root apex, the cyto-histological state and the physiological role of the different zones, and the embryogeny and differentiation of the root apex.

Analysis And Classification Of The Structural Net

Root apical organization of Pteridophytes—Esau (1953, 1965) mentions that these fall into two : one type exemplified by the root apices of Equisetaceae, Polypodiaceae and Marsileaceae, with a single tetrahedral apical cell from

---

The author is thankful to his Colleagues, Dr. B. D. Deshpande, and Dr. S. Bhambe and to his wife and co-worker, Dr. Ambuja Pillai, for going through the manuscript critically, for offering many helpful suggestions and for much help in the preparation of the manuscript.

which all other tissues are supposed to arise initially (Bower 1889, 1890, 1935). In the early stages of the development of the root tissues, this single cell is the source of all other cells and tissues. But this single apical cell does not remain perpetually active or totipotent (D'Amato and Avanzi 1965).

In the second type, exemplified by the root apices of Marattiaceae, a single tier of cells cuts off all other tissues of the root.

Bhambie (1963) reported a different organization at the root apices of *Isoetes coromandelina, I. engelmannii* and other species. These roots showed two tiers of initials, one giving rise to the stele in the centre and the other in the shape of an inverted cup, giving rise to the cortex, epidermis, columella and the peripheral region of the root cap. Recently, Bhambie and Rao (1971) have observed a single pyramidal cell in both young and old root apices of *Lygodium flexuosum, Microsorium alternifolium, Actiniopteris radiata* and *Nephrolepis exalta*. As the root grew older, the apical cell became prismatic and the surrounding cells highly cytoplasmic and mitotically active.

In *Osmunda regalis, Ophioglossum costatum* and *Ophioglossum nudicaulis* the young root apices showed one pyramidal cell but more were observed as the root grew older. At the apices of older roots these became prismatic.

The root apices of *Angiopteris evecta* showed a group of columnar prismatic cells surrounded by smaller meristematic cells.

On studying the root apices of *Isoetes coromandelina* again and *Isoetes panchananii*, they observed the mature root apex to have a common group of initials from which both root cap and root body originated.

Root apical organization of Spermatophytes.—The earliest reference to the study of root apical organization of higher plants is by Nicolai (1865). This was an era which was dominated by the "apical cell" theory of Naegeli (1845). Nicolai found the growing point of Spermatophyte roots to be composed of a group of cells in contrast to the single apical cell. Naegeli and Leitgeb (1868) also reported an irregular meristem. Thus, it was recognized that the root apices of higher plants were different from those of the cryptogams.

During the latter half of the nineteenth century workers studied mainly the cell net at the root apices with a view to correlating them with the apical cell on the one hand, and on the other, to classify the different structural types. Janczewski (1874), Treub (1876), Flahault (1878), Eriksson (1878), Schwendener (1882) and De Bary (1884) are the few names which we come across in this era. All of them tried to clarify and classify the structural organization at the root apices. They were influenced by another theory on shoot apical organization which Hanstein (1868) had propounded, namely, the "histogen theory". They tried to fit their observations on the cell nets at the root apices into this alternative theory when the number of initials was more. Janczewski (1874) added a fourth histogen, the calyptrogen, to the three proposed by Hanstein, as far as the roots are concerned.

Van Tieghem and Douliot (1888) reported the results of their extensive studies on the development of lateral roots. They called the Monocotyledons *Liorrhiz* and the Dicotyledons *Climacorrhiz* on the basis of the development of the root cap. They also tried to analyse their observations on the basis of the histogen theory and used the terminology of Hanstein. Though Hanstein's theory floundered to a great extent in the interpretation of the structure of the shoot apices, it was of use, with suitable modifications, in the interpretation of the structural net of root apices.

This interest in the analysis of the structural net continued till the middle of this century, though the interest was not consistently kept up. In the second decade of this century we meet with the publications of Kroll (1912), Haberlandt (1914) and Schüepp (1917). The latter introduced another concept, the "Körper-Kappe'' concept in the analysis of the cell net in the roots. This concept was incorporated in his review of 1926, which Esau has cited (1953, 1965). This concept was used by Wagner (1939) in analysing the ontogeny of the root cap in several plants and establishing the pattern of growth of cells by T-wall formation. Clowes (1950) has also used it in analysing the cell net at the root apices of *Fagus sylvatica*. Pillai (1963, 1964, 1966), Pillai and Pillai (1961a, b, c, d, e) and Pillai et al (1961, 1965a,b, c) have used this concept along with the histogen concept in analysing the root apical structures they investigated.

The fourth decade of this century records a resurgence of interest in the study of the root apices of Spermatophytes. Guttenberg (1940) postulated that there is a single "central cell" at the root tip, recalling the Naegelian hypothesis. Brumfield (1943) proposed a small group of three cells constituting the "initial group", which is more an adaptation of the Hansteinian hypothesis, on the basis of induced chimeras. However, others like Reeve (1948) could not distinguish any zonation at the root apices of *Pisum*, while Popham (1955) reported a transversal meristem. Controversies about the presence or absence of the central cell or layered initials were making their appearance. Pillai and Pillai (1961a) reported a "central group" in the apices of those roots with unstratified initials. As can be seen, most of these studies were directed towards analysing the cell net and classifying them structurally. Even though by this time Foster (1939) had emphasized the importance of studying the physiological state of the cell contents while formulating the cytohistological zonation theory, workers of root apical organization do not seem to have recognized the same at that time. As a matter of fact, the studies which were undertaken by us during the early days (Deshpande 1960, 1961; Pillai and Pillai 1961a, b, c, d, e; Pillai et al 1961, 1965a, b, c) were all directed primarily towards analysing the cell net, though the cytohistological aspect also received attention.

Pillai (1963, 1964, 1966) made a detailed study of the root apical organization of 33 species of Gymnosperms. She found that the organization of the root apices of the Cycads and *Ginkgo biloba* she studied was one type, that of the Conifers another, and that of *Ephedra foliata* a third. The apical organization of the roots of *Cycas revoluta*, *Cycas circinalis*, *Zamia* sp. and *Ginkgo biloba* showed a hemispherical or inverted cup-shaped generative centre with the bottom of the cup forming the stelar pole and columella and the initials for the cortex are the sides of the cup. The initials forming the side of the cup swing outwards in an arch. The cells at the distal part of this arch, by Kappe divisions, give rise to the peripheral region of the cap, while the cells in the proximal region give rise to the hypodermis. A single layered epidermis or rhizodermis covers the root.

According to her (1964) at the root apices of 27 species of Conifers there is an organization which could be analysed into four zones basing on the cell lineages. At the centre is a group of central initials from which distally the columella and proximally the stele arise. The stelar initials exhibit proximally Körper type of divisions whereas the columella initials exhibit mainly the rib meristem type of division. The central initials are covered by the initiating zone for the cortex and the peripheral region of the root cap, in the form of a hollow cup' through which the stele-columella complex passes. Buchholz and Old (1933) designated it as the "calyptro-periblem" while Guttenberg (1961) calls it the "periblemo calyptrogen". Its cell files exhibit Kappe divisions widening distally and producing the root cap while proximally they mature into the cortex. These observations do not support Schopf's (1943) interpretation that "the more lateral cells of the column shift polarity abruptly from the axis alignment and contribute to the peripheral region of the cap", an interpretation accepted by Allen (1946, 1947a, b) and Wilcox (1950, 1954).

The third type of organization observed by Pillai (1966) is that at the root apex of *Ephedra foliata*. Here she found a distinct tier of initials for the stele in contrast to the report of a transversal meristem by Deshpande and Bhatnagar (1961). Distal to the stelar initials are the initials for the columella. Surrounding them, as in the case of the Conifer roots, she found a common initiating region for the cortex and the peripheral region of the cap. The structures described above were analysed by following the Körper-Kappe hypothesis of Schüepp (1917).

Working on the root apical organization of some members of the Amaryllidaceae and Liliaceae, Deshpande (1960,1961) reported four types of configuration at the root apices of these plants. One type has all common initials as met with in *Aloe vera, Hemerocallis flava, Eucharis grandiflora* and *Amaryllis belladona*. In the second type the stele has separate initials and all other regions arise from another generative centre, as in *Crinum latifolium* and *Agapanthus africanus*. In the root apices of *Haemanthus coccineus, Polygonatum oppositifolium* and *Ruscus hypophyllum* occurs the third type with calyptrogen and plerome independent and a common initiating zone, the dermoperiblem, for the epidermis (rhizodermis) and cortex. The fourth type is exemplified by the root apex of *Zephyranthes tubispatha, Sansevieria hahnii* and *Sansevieria thyrsiflora* with four independent initiating zones, one each for the root cap, epidermis, cortex and stele.

Pillai and Pillai (1961a, b, c, d, e) and Pillai et al (1961) studied the root apical organization of a number of species belonging to the families Musaceae, Cannaceae, Marantaceae, Zingiberaceae, Palmeae and Xyridaceae of Monocotyledons. They also found four different types of configuration: (1) with a common initiating zone for all regions of the root, as in *Musa paradisiaca, Musa sapientum, Ravenala madagascariensis, Heliconia illustris, Zingiber officinale, Cocos nucifera, Phoenix sylvestris, Phoenix dactylifera, Cyrtostachys lakka* and *Areca lutescens*. (2) with separate initials for the stele, a dermoperiblem complex, and root cap, as is most commonly met with *e.g.*, in *Curcuma longa, Curcuma* *aromatica, Costus speciosus, Hedychium coronarium, Kaempferia galanga, Kaempferia rotunda, Canna indica, Canna edulis, Calathea zebrina, Stromanthe sanguinea, Phrynium capitatum, Maranta variegata, Maranta major, Alpinia calcarata, Maranta zebrina, Elettaria cardamomum, Calamus rotang, Xyris anceps,* and *Xyris schoenoides.* Pillai and Sachdeva (1960) have also described this type at the root apex of *Sorghum vulgare.* (3) with four tiers of initials, one for the stele, two for the cortex, of which the outer gives rise to the epidermis and hypodermis, and the inner to the rest of the cortex, and a fourth to the root cap. This was found at the root apex of *Maranta arundinacea.* (4) with a dermocalyptrogen outside and a common zone for the stele and cortex. This was exhibited by the root apex of *Pritchardia pacifica.*

The distinction of the root cap into the peripheral region and the columella, and the concept of a separate initiating layer for the latter, the "columellogen", was put forward by Pillai and Pillai (1961a) during these studies.

Pillai et al (1965a, b, c) have also studied the root apices of some Dicotyledons belonging to families ranging from those considered more or less primitive like Ranunculaceae and Proteaceae to highly evolved ones like Compositae. They found the following types of root apical structures: (i) with a common group of initials for all tissues of both the root body and root cap, as in *Gossypium hirsutum, Althea rosea, Manihot utilissima, Grevillea robusta, Amaranthus gangeticus, Piper nigrum, Gaillardia picta. Acroclinum luteum,* and *Echinops echinata.* (ii) with separate initials for the stele, cortex and a common initiating zone for the epidermis and root cap, as met with at the root apices of *Delphinium ajacis, Eriodendron anfractuosum, Zinnia elegans, Brachycome iberidifolia, Tridax procumbens, Limnanthemum cristatum* and *Brassica nigra.* Chandhoke (1968) has also observed this type of organization at the root apices of *Calligonum polygonoides, Rumex vesicarius* and *Fagopyrum esculentum.* (iii) with separate initials for the stele, cortex, hypodermis and a common initiating zone for the epidermis and root cap. This type is rare and was met with at the root apex of *Jussiaea repens* and *Centaurea cyamus.*

An important feature these studies brought out was the configuration of the two parts constituting the "root cap". This is composed of two distinct portions in many of the root apices, with their cell lineages arranged in two distinct ways. One is the central "columella" and the other the "peripheral region of the cap". The former is composed of more of less vertical files of cells which are generated by the transverse divisions of initials located distal to the stelar pole. The number of such files is more often the same from tip to the generative centre, though occasional T-divisions may occur with the capital of the T toward the distal part of the root cap. The peripheral region, on the other hand, is made up of files of cells which curve from the flanks towards the pericolumnar region exhibiting T-divisions with the capital of the T towards the flanks. This results in increase in the number of cell files from the flanks towards the pericolumnar region.

This situation posed a problem as to the use of the word "calyptrogen" (not in the strict sense of Hanstein but in the sense of the initials giving rise to the cap as a whole) for understanding the formation of two different types of cell lineages.

An understanding of this is possible only by studying their development from early embryo onwards. This and certain other aspects like the opening out of the meristems from the root body to the root cap (see later) stressed the need and importance of developmental studies.

One of the important differences between the root apices of the Monocotyledons and Dicotyledons is in their mode of root cap formation, particularly the peripheral region of the cap. Among the Dicotyledons the root cap and root epidermis have a common origin so that the root body and root cap are connected even from the embryo stage. In Monocotyledons the epidermis and cortex have very often a common origin whereas the root cap has a separate origin. Since most of the monocotyledonous plants studied were vegetatively propagated, developmental stages could be traced only during lateral root development. This showed that the cap arose by the proliferation of the endodermis of the mother root whereas the root body developed from the pericycle (Pillai & Pillai 1961 b, c). It would be interesting to study the embryo stages in the Monocotyledons which set seeds.

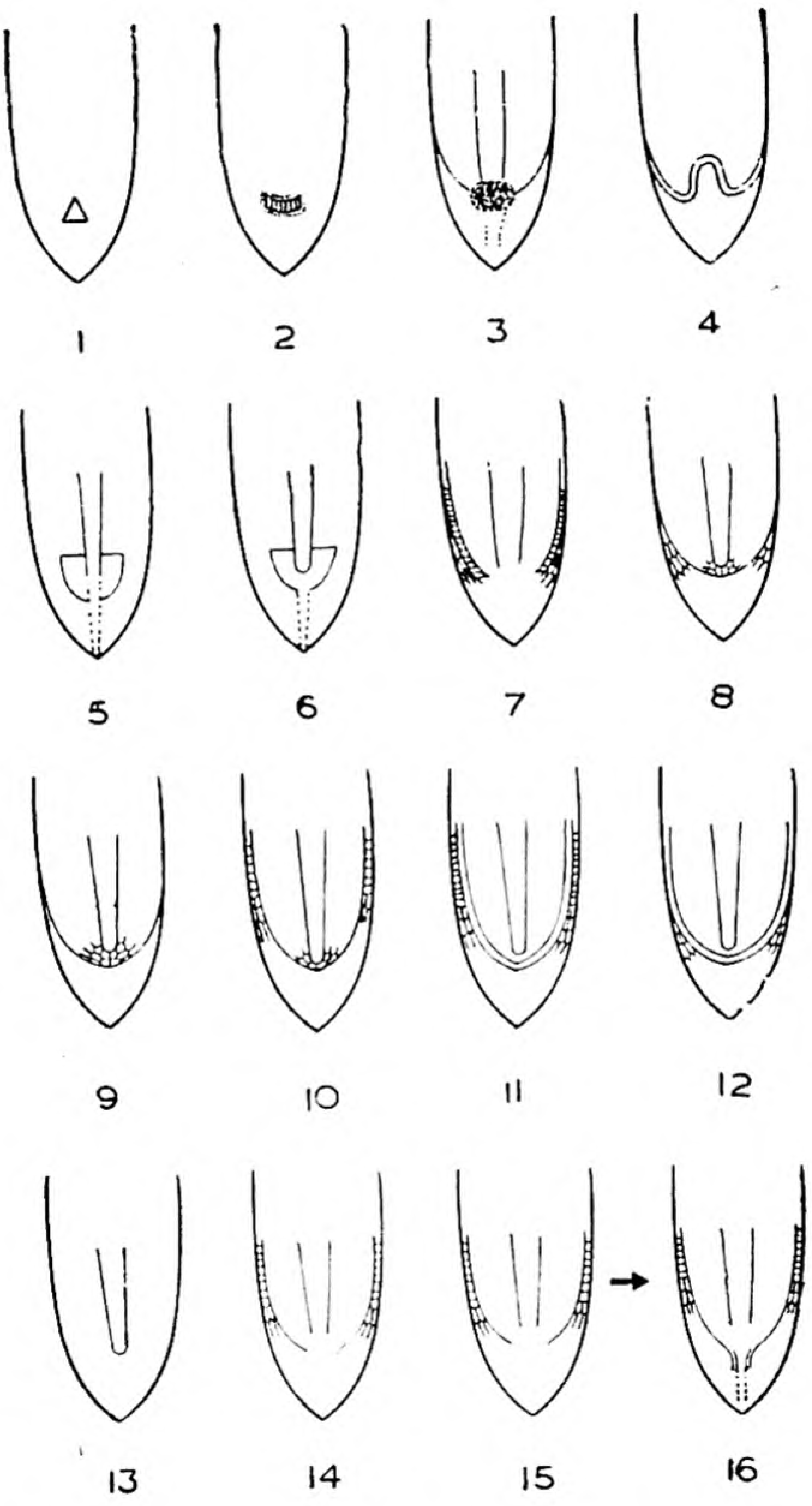
It has been found in some studies (unpublished) on the root apices of members of the Gramineae that not only is the root cap separate from the root body but also that there is a layer of mucilaginous matrix separating them. A question which normally arises in such cases would be as to how the cells of the root cap get their metabolite requirements from the root body. This study would be rewarding.

Our studies here on 12 species of Pteridophytes, 33 species of Gymnosperms, 44 species of Monocotyledons and 23 species of Dicotyledons have brought out the following types of structrual organization in the roots of the different plants:— In Pteridophytes three types, (i) with a single apical cell (Fig. 1) (ii) with a plate of a few initial cells (Fig. 2), and (iii) with a group of inconspicuous apical initials (Fig. 3). In Gymnosperms also three types of organization are met with, (1) the Cycad type, with an inverted cup-shaped initiating region (Fig. 4), (2) the Conifer type, with a mortar and pestle type of arrangement of apical initials, where the pestel passes through the hollow base of the mortar (Fig. 5), and (3) the *Ephedra* type, with discrete initials for the stele and columella—the pestle with two separate regions—and the mortar with its hollow base surrounding them (Fig. 6). In Angiosperms eight types are met with. Type 1 with common group of initials for all regions of the root. (Fig. 7). Type 2 with separate root cap and stelar initials and a common dermoperiblem (Fig. 8). Type 3 with separate root cap and stelar initials and a two-tiered dermoperiblem complex (Fig. 9). Type 4 with separate initials for the stele and cortex and a common dermocalyptrogen (Fig. 10). Type 5 with discrete initials for the stele, another for cortex except hypodermis, a third for the hypodermis, and a fourth the dermocalyptrogen (Fig. 11). Type 6 with discrete initials for the cap, epidermis, cortex and stele (Fig. 12). Type 7 with discrete initials for the stele and a common generative centre for all other tissues (Fig. 13). Type 8 with a dermocalytrogen outside and a common generative centre for all other tissues (Fig. 14).

SUGGESTIONS ON THE EVOLUTIONARY TREND IN ROOT APICAL STRUCTURES.

There are very few views mentioned about this aspect in the literature. The few which have been put forward relate to the root apices of Angiosperms, which will be mentioned later.

Bhambie and Rao (1971) have put forward a suggestion on the evolution of the root apical structures in the Pteri-



Figs. 1-16. Figs. 1-3. Different types of organization at the root apices of Pteridophytes. Figs. 4-6. Same of Gymno sperms. Figs. 7-14. Same, Angiosperms. Figs. 15, 16 Phenomenon of opening out of the root body into the root cap.

diophytes. According to them the root apices with a single conspicuous apical cell as found in the leptosporangiate ferns *Actiniopteris, Lygodium,* and *Microsorium* form the most primitive, followed by those of the eusporangiate ferns like *Angiopteris, Osmunda* and *Ophioglossum* with a plate of initials. The most advanced type is met with in the fern allies *Isoetes* species with a group of inconspicuous initials. No other reports on the evolutionary trend in the root apical structures of Pteridophytes are known to the author.

Haberlandt (1914) had put forward a scheme on the evolution of the root apical structures in the Spermatophytes based on the types of root cap formation. According to this, the simplest type is the one where the root cap arises from a separate meristematic layer, the calyptrogen. The next advance is to the type where the dermocalyptrogen occurs. The third step is where the protoderm and the other cortical layers also contribute to the cap. The point of origin of the cap becomes more and more deep-seated in the more advanced types, ultimately reaching a climax where the meristem of the root cap and root body proper coalesce to form a common meristem.

Voronin (1956) has, on the other hand, considered the apical structure with common initials for all regions to be the most pirmitive phylogenetically.

Pillai (1966) has made a tentative suggestion on the line of evolution of the root apical structures within the Gymnosperms. Starting from the structure with a common or undifferentiated type of generative centre in the Cycad and *Ginkgo* root apices, the line advances to the structure in the Conifer root apices where the stele and columella arise from one set of initials and the cortex and peripheral part of the root cap from another. The line culminates in the *Ephedra* type with a separate tier for the stele, another set of initials for the columella, and a third set for the cortex and peripheral region of the root cap.

Pillai (1963) has made a suggestion on the evolutionary trend in the root apical structures of the Scitaminales. The most primitive is the one with the common group of initials for all regions of the root cap. This is followed by the one with separate root cap and stelar initials and the common dermoperiblem complex. The last and most evolved in this series is the one with separate cap and stele and a two-tiered dermoperiblem complex.

Pillai et al (1965b) have suggested a more or less similar evolutionary trend in the root apical structures. of the Compositous plants. The most primitive they consider to be the type with no specialization in the activity of the initials. The next higher structure is the one with separate initials for the stele and cortex. The most evolved in this series is the one with discrete initials for the stele inside and hypodermis outside with another tier between them which gives rise to the rest of the cortex.

The trend of evolution of the root apical structures appears to be different in the Pteridophytes and Spermatophytes. The trend in the former appears to be from the simple to the complex whereas that in the latter appears to be from the one with common group of initials to those with more stratified initials. However, the occurrence of more than type in members of the same family would caution us in accepting the interpretation based on observations of a few structures. Much more root apices of many plant species will have to be investigated to establish the validity or otherwise of these suggestions.

STUDY OF THE CYTO-HISTOLOGICAL STATE OF THE ROOT APICES

Clowes (1956a, b) brought out the fact that there are differences in the physiological state of the cells at the root apex and that all cells located at the apex are not functionally similar. By using differential staining and labelled compounds he showed that the cells at the extreme tip of the root apex (excluding those of the root cap) were in a state of comparative repose and that the region of real meristematic activity was in the proximal cells surrounding the quiescent centre. During their studies of monocotyledonous root apices, Deshpande (1960, 1961) and Pillai and Pillai (1961 a, b, c, d, e) and Pillai et al (1961, 1965 a, b, c) also observed this difference by using differential vital stains. Pillai and Pillai (1961a) called the region of greater meristematic activity surrounding the quiescent centre as the Meristematic zone". Support for the quiescent centre hypothesis came from the studies of Jensen (1957, 1958), Hejnowicz (1959), Thomas (1967) and others. The quiescent centre was not evident in the young root and it has been reported as appearing when the root begins to grow thicker and older. In a series of investigations using labelled precursors of DNA, Clowes (1956a, b; 1958a, b; 1959a, b; 1961; 1965; 1967) and Thompson and Clowes (1968) have further confirmed the presence of the quiescent centre. Pillai and Sachdeva (1960) and Pillai and Pillai (1962) have supported the quiescent centre concept based on the responses of the quiescent centre and the surrounding meristematic zone to some surgical experiments. Pillai et al (1965b) had studied the number of mitotic figures in the quiescent centre and meristematic zone of the roots of *Cosmos bipinnata* at one hour intervals during a day. They found that the number of divisions was much less in the quiescent centre as compared to the meristematic zone at all times. They also found that there was a rhythm in the divisions during a day. D' Amato and Avanzi (1965) and Bhambie and Rao (1971) found that the quiescent centre may develop even in roots like those of a fern with a single tetrahedral apical cell, and those in which there is a plate of bigger cells, and that the quiescent centre may develop as the root grows older and thicker. Some recent reports of the development of the quiescent centre in roots are those of Raju et al (1964) and Byrne and Heimsch (1970 b). The latter authors report the quiescent centre to be present in the embryo of *Malva sylvestris* seeds. More studies on the development of the quiescent centre and surrounding tissues in more roots and the roles of the quiescent centre and surrounding tissues would be helpful in determining their respective physiological roles in growth and regeneration of the roots.

DEVELOPMENT OF APICAL STRUCTURES DURING EMBRYOGENY

Another aspect of the study of root apical organization is to trace the development of the initiating regions of the root apex from the early embryo onwards. Padmanabhan (1967, 1968) has reported observations on the early development of the tissue zones like the dermal layer, cortex and stele in *Epithema carnosum* and *Tridax procumbens*. However, he has not traced the development of the different initiating regions of the root apex, which if he had done, would have enriched our knowledge of root apex ontogeny. Such studies on tracing the development of the different regions at the root apex, were undertaken by Spurr (1949), Guttenberg et al (1954a, b; 1955; 1960), Mahlberg (1960) and others.

On the basis of their studies Guttenberg found that the root apical structures can be brought under two broad categories, the "closed" where the apex has a configuration with layered initials and which does not change during ontogeny and the "open" where the early embryo shows a tiered configuration which changes as the embryo grows and the cell files extend from the root body to the root cap. This extension is effected by "knee-like" formations of cell files which proceed from around the zone containing the initials for the columella files. According to Guttenberg (personal communication) these contributions of the root body to the root cap occur not only through the knees but also from the proximal end of the columella, as a result of which all the original columella files are pushed distally and in course of time disappear. Because of this Guttenberg (1961) feels that there is no quiescent centre of a permanent nature and he calls the region as "intermittent centre". However Pillai and Sukumaran (1969) found some differences from Guttenberg's interpretation in their studies on *Cyamopsis tetragonoloba* embryos. In the young embryos they found common initials for all regions except the peripheral region of the root cap. As the embryo grows older, the outermost cortical files, which converge around the proximal end of the columella files exhibit "knees" and proceed into the cap but surrounding the original columella files and producing secondary columella files. The original columella files were not disturbed (Fig. 15, 16). Pillai et al. (1965c) had noted the knee formation in some root apices of *Grevillea robusta* but could not trace the ontogeny. Byrne and Heimsch (1970a) have recently reported on the change from 'closed' to 'open' root apical structures in *Malva sylvestris*, They studied the root apical structures from that of the fully developed embryo onwards. In the fully developed embryo they report discrete initials for the central cylinder inside, a layer of secondary columella initials outside it and a ring of cortical initials surrounding them. However the development of these layers has not been traced from earlier embryos, without which it is difficult to say that they have arisen in the positions these authors have indicated. Normally, when the initials for the primary columella are found they can be expected to continue to be active in cutting off cells for the columella and there will be no necessity for another plate of initials to be developed for replacing the active initials of the primary columella. Also, the report does not take into consideration what the state of the cortical initials was before the formation of the secondary columella initials. Studies starting from earlier embryos would bring out these points which are not now clear. Trivedi (1969) made such a study in *Prosopis juliflora* and found that the peripheral region of the root cap was constituted of cell files from both the dermocalyptrogen and the outer regions of the cortex at the late globular stage of the embryo onwards. This is a feature which is more akin to the formation of the cortex and cap flanks in the Conifer and *Ephedra* root apices (Pillai 1964, 1966). At the torpedo stage of the embryo the cap flanks could be distinguished into parts, the outer arising from the protoderm and the inner from the outer layers of the cortex. However, all the cortical layers are not involved in this and those concerned with the formation of the cortex can be distinguished by the Körper type of divisions which they exhibit. Trivedi could distinguish the columella initials even at the globular stage. They divide anticlinally (transversely) with occasional vertical divisions to become wider. At the torpedo stage of the embryo the cells located about 5 to 6 cells distal to the stelar promeristem and abutting on the columella divide obliquely and transversely resulting in a bulge around the columella. Later, divisions occur both distal and proximal to the bulge and thus the secondary columella arises. The cortical files around the promeristem also exhibit the knees resulting in contributing secondary files around the primary columella.

WHAT IN FUTURE ?

The points raised in the preceding pages bring to our attention the lacunae which exist in our knowledge about root apical organization.

As at present we have information about the structural net at the root apices of only a few species of plants. More of them have to be studied and information collected, particularly about primitive orders on the one hand, and advanced ones on the other.

Though studies till date have shown that there is no form of structural net common to all members of a family, further more detailed studies may be directed towards this aspect and towards correlating the results with taxonomic or other characters.

We are not sure whether any correlation exists between the root apical sructures and other anatomical and embryological features of plants. Studies may be specially directed towards particular groups of plants which may bring out these correlations. In this connection, it may be borne in mind that the shoot apical organization has been used in some cases to clear up systematic disputes.

Only meagre information is available at present about the early embryogeny and histogenesis. As has already been brought out, such studies would give better developmental pictures of the different regions of the root.

Has the opening out of apical initials any special significance? We do not have any idea of the significance of tiered initials in some and common initials in others though both result in a mature structure which serves the same purpose. What are the physiological influences which enable the differentiation of the various zones of the root body? Is there any physiological role for the quiescent centre other than what we know now?

Some of these questions are being raised so that future workers may try to find answers to them. With the advances in techniques and methods in studying living objects at not only the organ level but even at the molecular level, we can hope that efforts may be directed towards understanding the causes underlying the behaviour of initials in set patterns and other points raised above, erelong.

REFERENCES

Allen, G.S. 1946. Embryogeny and development of the apical meristems of *Pseudotsuga*. I. *Am J. Bot.* **33:** 666–677.

——. 1947a. Embryogeny and development of the apical meristems of *Pseudotsuga*.-II. *Am. J. Bot.* **34:** 73–80.

——. 1947b. Embryogeny and development of the apical meristems of *Pseudotsuga*.-III. *Am. J. Bot.* **34:** 204-211.

Bhambie, S. 1963. Studies in Pteridophytes-IV The development, structure and organization of root in *Isoetes coromandelina* L. *Proc. Indian Acad. Sci.* 58B: 135-164.

——., and C.G.P. Rao. 1971. Studies in Pteridophytes-IX. The root apex organization in some Pteridophytes. (in course of publication).

Bower, F.O. 1889. The comparative examination of meristems of ferns as a phylogenetic study. *Ann. Bot., Lond.* **3:** 305-392.

——. 1890. Is the eusporangiate or the leptosporangiate the more primitive type in the ferns ? *Ann. Bot., Lond.* **5:** 109-134.

——. 1935. *Primitive Land Plants.* MacMillan and Co., London.

Brumfield, R.T. 1943. Cell lineage studies in root meristems by means of chromosome rearrangements induced by X-rays. *Am. J. Bot.* **30:** 101-110.

Buchholz, J.T., and E.M. Old. 1933. The anatomy of the embryo of *Cedrus* in the dormant stage. *Am. J. Bot.* **20:** 35-44.

Byrne ,J. M., and C. Heimsch 1970a. The root apex of *Malva sylvestris*.I. Structural development. *Am J. Bot.* **57:** 1170-1178.

——, and——. 1970b. The root apex of *Malva sylvetsris*.-II. The quiescent centre. *Am. J. Bot.* **57:** 1179-1184.

Chandhoke, K. R. 1968. *Morphological and Anatomical Studies of Certain Members of Polygonaceae.* Ph.D. Thesis, Birla Inst. Tech. Sci., Pilani.

Clowes F.A.L. 1950. Root apical meristems of *Fagus sylvatica. New Phytol.* **49:** 248-267.

——. 1956a. Localization of nucleic acid synthesis in root meristems. *J. exp. Bot.* **7:** 307-312.

——. 1956b. Nucleic acids in root meristems of *Zea. New Phytol.* **55:** 29-34.

——. 1958a. Development of quiescent centre in root meristems. *New Phytol.* **57:** 85-87.

——.1958b. Protein synthesis in root meristems. *J. exp. Bot.* **9:** 229-238.

——.1959a. Reorganization of root meristems after irradiation. *Ann. Bot. N. S.* **23:** 205-210.

——.1959b. Adenine incorporation and cell division in shoot apices. *New Phytol.* **58:** 16-19.

——.1961. *Apical Meristems.* Blackwell, Oxford.

——.1965. Meristems and the effect of radiation on cells. *Endeavour.* **24:** 8-12.

——.1967. The quiescent centre. *Phytomorphology.* **17:** 132-140.

D'amato, F., and S. Avanzi. 1965. DNA content, DNA synthesis and mitosis in the root apical cell of *Marsilea strigosa. Caryologia* **18:** 383-394.

De Bary, A. 1884. *Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns.* Clarendon Press, Oxford.

Deshpande, B. D. 1960. Root apical meristems in Monocots-I. Root apex organization in some members of the Amaryllidaceae. *J. Indian bot. Soc.* **39:** 126-132.

——.1961. Root apical meristems in Monocots-II. Root apical organization in some members of the Liliaceae. *J. Indian bot. Soc.* **40:** 535-541.

——, and Prabha Bhatnagar. 961. Apical meristems of *Ephedra foliata. Bot. Gaz.* **122:** 279-284.

*Eriksson, J. 1888. Über das Urmeristem der Dikotylen Wurzeln. *Jb. wiss. Bot.* **11:** 380-436.

Esau, Katherine 1953. *Plant Anatomy.* (Ist. edition John Wiley and Sons, New York.

——, ——. 1965. *Plant Anatomy.* (2nd edtion (John Wily.) New York.

Fahn, A. 1967. *Plane Anatomy.* New York.

Flahault, C. 1878. Recherches sur l'accroissement terminal de la racine chez les phanerogames. *Ann. Sci. nat., Bot.* **6:** 1-168.

Foster, A. S. 1939. Problems of structure, growth and evolution in the shoot apex of seed plants. *Bot. Rev.* **5:** 454-470.

Guttenberg. H. von. 1940. Der Primäre Bau der Angiospermenwurzel. In: K. Linsbauer's *Handbuch der Pfianzenanatomie* Gebruder Borntraeger, Berlin.

——. 1960. Grundzüge Histogenese der höherer Pflanzen-I. Die Angiospermen. In : K. Linsbauer's *Handbuch der Pflanzenanatomie* Gebruder Borntraeger, Berlin.

——.1961. Grundzuge Histogenese der höherer Pflanzen-II. Die Gymnospermen. In : K. Linsbauer s *Handbuch der Pflanzenanatome.* Gebruder Borntraeger, Berlin.

——,J. Butrmeister and H. J. Brosel. 1955 Studien uber die Entwicklung des Wurzelvegetationspunktes der Dikotyledonen-II. Planta, **46:** 179-200.

——,H. R. Heydel, and H. J. Pankow. 1954a. Embryologische Studien an Monokotyledonen-I. Die Enstehung der Primärewurzel bi *Poa annua. Flora* **141:** 298-311.

——, ——, and ——. 1954b. Embryologishe studies an Monokonyledoner-II (Die Entstehung der Primärewurzel bi *Allium giganteum. Flora* **141:** 476-500.

Haberlandt,G. 1914. *Physiological Plant Anatomy* Mac Millan and Co., London.

*Hanstein, J. 1868. Die Scheitelzellgruppe in Vegetationspunkt der Phanerogamen. Festschr. Neiderrhein. Gessell Natur-und Heilkunde. 109-143.

Hejnowicz, Z. 1959. Growth and cell division in the apical meristems of wheat. *Physiol. Pl.* **12:** 124-138.

*Janczewski, E. De. 1874. Recherches sur l'accroissement terminal dans racines les phanerogams. *Ann. Sci. nat. Bot.* **20:** 162-201.

Jensen,W.A. 1957. The incorporation of C-14 adenine and 14 C-phenylalanine by developing root tip cells *Pro cnatn Acad Sci., U.S.A.* **43:** 1038-1046.

——.1958. The nucleic acid and protien component of root tip cells of *Vicia faba* and *Allium cepa. Expl. Cell Res.* **14:** 575-583.

Kroll, G.H. 1912. Kritische Studie uber die Verwertbarkeit der Wurzelhaubentypen fur die Entwicklungsgeschichte. *Bot. Centrabalt Beihefte.* **28:** 134-158.

Mahlberg, P. G. 1960. Embryogeny and histogenesis in *Nerium oleander*-I. Organization of primary meristematic tissues. *Phytomorphology* **10:** 118-131.

*Nägeli, C. B. 1845. Wachathumgeschichte der Laubund Leermoose. Z. *wiss. Bot.* **2:** 138-210.

——, and H. Leitgeb. 1868. Entstehung and Wachsthum der Wurzeln. *Beitr. wiss. Bot.* **4:** 73-160.

*Nicolai, P. 1865. Das Wachsthum der Wurzelschriften der physikal. Okonom Gesselsch in Konningsberg, VII,P.73.

Pillai, Ambuja. 1963. Root apical organization in Gymnosperms.——Some Cycads and *Ginkgo biloba*. *Proc. Indian Acad. Sci.* **57:** 211-222.

——,——. 1964. Root apical organization in Gymnosperms—Some Conifers. *Bull. Torrey bot. Club* **91:** 1-13.

——,——. 1966. Root apical organization in Gymnosperms—*Ephedra foliata*, with a suggestion on the possible evolutionary trend of root apical structures in Gymnosperms. *Planta* **70:** 26-33.

Pillai, S. K. 1963. A tentative suggestion on the evolutionary trend in the root apical structures in members of the Scitaminales. *Phyton* (Redactio) **10:** 253-258.

——, and Ambuja Pillai 1961a. Root apical organization in Monocotyledons—Musaceae. *J. Indian bot. Soc.* **40:** 444-455.

——, and ——. 1961b. Root apical organization in Monocotyledons. Cannaceae. *J. Indian bot.* **40:** 645-656.

—— and ——. 1961c. Root apical organization in Monocotyledons-Marantaceae. *Proc. Indian Acad. Sci.* **B53:** 302-317.

—— and ——, 1961d. Root apical organization in Monocotyledons Palmeae. *Proc. Indian Acad. Sci.* B.**54:** 218-233.

——, ——. 1961e. Root apical organization in Monocotyledons Xyridaceae. *Proc. Indian Acad. Sci. B.* **54:** 234-240.

——, ——. 1962. Reactions of root apices to some surgical experiments. *J. Indian bot. Soc.* **41:** 148-155.

—— . and Susheila Suchdeva, 1960. Effect of some surgical excisions on the regeneration of the root apex of *Sorghum vulgare*. *Curr. Sci.* **29:** 233-234.

—— and K. Sukumaran. 1969. Histogenesis, apical meristems and anatomy of *Cyamopsis tetragonoloba*. *Phytomorphology* **19** 303-312.

——, Ambuja Pillai and Susheila Sachdeva 1961. Root apical organization in Monocotyledons—Zingiberaceae. *Proc. Indian Acad. Sci.* **B53:** 240-256.

——, A. Pillai, and P. Girijamma. 1965a. Apical organization of the roots of Dicotyledons-I. Root apices of some members of Ranunculaceae, Malvaceae, Bombacaceae, and Euphorbiaceae. *Proc. Raj. Acad. Sci.* **8:** 43-59.

——, P. Vijayalekshmi, and Omana M. George. 1965c. Apical organization of the roots of Dicotyledons -II. Root apices of some members of Proteaceae, Cruciferae, Piperaceae, Amaranthaceae, Onagraceae, Gentianaceae and Scrophulariaceae. *Proc. Indian Acad. Sci.* **B61:** 267-276.

——, Omana M. George, and P. Vijayalekshmi. 1965b. Apical organization of the roots of Dicotyledons-III. Root apices of the Compositeae. *Proc. Indian Acad. Sci.* **B61:** 296-308.

Popham, R.A. 1955. Zonation of primary and lateral roots of *Pisum sativum*. *Am. J. Bot.* **42:** 267-273.

Raju. M.V.S., T.A. Steeves, and J. M. Naylor 1964. Developmental studies on *Euphorbia esula*. Development of long and short roots. *Can. J. Bot.* **42:** 1615-1628.

Reeve, R.M. 1948. Late embryogeny and histogeneais in *Pisum*. *Am. J. Bot.* **35:** 591-602.

Schopf, M. 1943. Embryology of *Larix*. III. *Biol. Mongr.* **19:** 3-97.

Schüepp, O. 1917. Untersuchungen uber Wachsthum und Formwechsel von Vegetationspunkten. *Jb. wiss. Bot.* **57:** 17-79.

——. 1926. Meristeme, In : K. Linsbauer, *Handbuch der Pflanzenanatomie*. Gebruder Borntraeger Berlin.

*Schwendener, S. 1882. Die Schutzscneiden und ihre Verstarkungen. *Abhandl. konigl. Akad. wiss., Berlin, Abh.* III: 1-75.

Spurr, A. R. 1949. Histogenesis and organization of the embryo in *Pinus strobus*. *Am. J. Bot* **36:** 629-641.

Thomas, D.R. 1967. The quiescent centre in cultured tomato roots. *Nature, Lond* **214:** 739.

Thompson, J. and F. A. L. Clowes. 1968. The quiescent centre and rates of mitosis in root meristem of *Allium sativum*. *Ann. Bot, N.S.* **32:** 1-13.

*Treub, M. 1876. *Le meristeme de la racine dans les Monocotyledones*. E.J. Brill Author Leiden.

Trivedi, M.L. 1969. *Certain Aspects of the Developmental Anatomy* of *Prosopis juliflora*. Ph. D. Thesis, Birla Inst. Tech. Sci., Pilani.

*Van Tieghem, P. *and H. Douliot. 1888. Recherches comparatives sur l origine des members endogenes, *Ann. Sci. nat., Bot.*, **8:** 1-661.

*Voronin, N. S. 1956. Of evoliustii Kornei ——Obshch Isp, priorody otd. biol. biul. **61:** 47.

Wagner, N. 1939. Über die Entwicklungsmechanik der Wurzelhaube und des Wurzelrippenmeristeme. *Planta*. **30;** 21-66.

Wardlaw, C. W. 1968. Morphogenesis in Plants—a contemporary study. Methuen and Co., London.

Wilcox, R. H. 1950. *The Regeneration of the Injure Root Systems in Noble fir, Abies procera*. Ph. D. Thesis, Univ. California, Berkeley.

——. 1954. Primary organization of the active and dormant roots of noble fir, *Abies procera*. *Am. J. Bot.* **41:** 812-821..

---

* Original not seen.

Adv. Pl. Morph. 1972 : 291–303

VEGETATIVE TO REPRODUCTIVE STATE OF APEX IN MONOCOTYLEDONS

By Y. S. Murty and V. Kumar

Department of Botany, Meerut University, and Meerut College, Meerut, India

Abstract

The problems of transition of vegetative apex into reproductive apex including the various factors causing this profound change in the initial steady state have been reviewed and discussed. The suggested lines for further investigation are also discussed as formulated by Wardlaw e. g. (a) general and specific gene action, (b) regulative and correlative factors mediated through the reaction system, (c) sequence of biochemical reactions and (d) biophysical factors. All these factors along with the external factors should be investigated fully. Before any experiment could be designed on these lines so as to have a complete understanding of this problem of selective and state differentation, sufficient anatomical grounds should be attained in various taxa to understand the various organisational components. From the discussion, it also becomes clear that the vegetative, transition and floral meristems may or may not differ much from each other in configuration etc., but all give rise to, perhaps growth centres with a rythm which is a complex of the reaction system that eludes our present comprehensions.

Introduction

The morphology of inflorescence and flower, homologies of the constituent organs, their origin and phylogeny, their inception and development in the individual plant, have afforded unrivalled scope for both theorists and experimentalists in this field to find uniformity in diversity. The inflorescence (or flower in case of terminal flowers) usually develops from a terminal or lateral or vegetative shoot apex and results in the culmination of activity of the particular meristem. Thus the floral apex unlike the vegetative apex, exhibits determinate growth like the leaf primordium. However, in others growth of the plant may end in flowering if all the terminal and lateral shoot apices are transformed into flowers or inflorescences. Incidentally, monocotyledons constitute the major part of this type of plants. They usually develop culm and die off after maturation or flowering.

As per classical view, the flower, in which the sepals, petals, stamens and carpels are successive organs is regarded as a compressed shoot. The diverse lateral organs of flower in the opinion of Goethe (1790) are different forms of an idealized lateral member — the leaf (see Arber, 1937). Grégoire (1938) on the other hand interpreted the floral apex as organ *Sui generis* (unique, not classifiable with others). The works of Philipson (1946, 1947a, 1947b, 1948a, b, and 1949) and others have, however, established that

---

This communication results from an inspiration the authors received from Professor Puri and his services in the cause of Floral Morphology while working in his laboratories under his kind guidance.

at flowering, the vegetative apex of main or lateral shoot undergoes various physiological and structural changes and becomes directly transformed into a reproductive apex of a flower or an inflorescence.

Another view which is supported by observations on grasses, (Sharman, 1942 a,b, 1947; Barnard, 1957a,b; 1958, 1961) and *Allium* (Jones and Emsweller, 1936) is that the carpels originate like leaves, whereas stamen primordia are bud like outgrowths. Again, some workers (Wilson and Just 1939) have envisaged a phylogenetic origin from a branched structure bearing microsporangia i.e. the telomes, and from fascicled type of stamens in Malvales, Guttiferae etc. (Van Heel 1966, 1969). Such observations laying emphasis on phylogenetic origin have led to the concept of Gonophyll (Melville, 1962, 1963 and Sterling, 1963). Melville (1963) argued that as the term 'flower' refers to a biological rather than morphological unit, it becomes applicable both to single flower and to condensed inflorescences. Puri (1964) after a careful analysis of this hypothesis and of objections raised by Melville against the classi cal concept has shown this to be an unconvincing and purely hypothetical consideration.

Several reviews have appeared on floral morphology especially by Bancroft (1935), Arber (1937), Wilson and Just (1939), Puri (1951, 1952), Douglas (1957), Barnard (1955, 1961) and Eames (1961).

The morphogenetic approach to the floral development is not a recent one and Payer as early as 1857 had shown how much could be learnt by simple observations about the transition from vegetative to flowering state, and the inception and development of floral organs. The morphological questions are now being reexamined from the developmental or morphogenetic stand point by Barnard (1955, 1957a,b, 1958), Eckert (1966). Murty and Kumar (1970, 1972) etc. (also see Cutter, 1965). Wardlaw (1957, 1961, 1965a, 1968) has suggested an approach to an understanding of the biology of differentiation by collecting sufficient information on:—

1. "Steady state of vegetative apex, the constitution, the distribution of growth in it, its organogenic activities and the temporal relationships".
2. "Incedences of factors stimulating or inducing chages in the distribution of growth in steady state of the apical reaction system".
3. "Serial evocation and specific activities of the genes or groups of genes".
4. "Factors involved in phenomenon of correlation determining the holistic and integrated development of flowering".

Heslop-Harrison (1964) also considers that each phase of floral development represents the expression of a different gene compliment. Cutter (1971) remarks this to be an "epigenetic" phenomenon, the expression of each set of genes depending on that of a preceeding set. This aspect is not well investigated yet, and therefore, not discussed in the present article.

According to the species and environmental factors, the vegetative apical meristem for a certain period of time during ontogenesis continues in a state of active growth and at regular intervals gives rise to regularly spaced leaf primordia often associated with bud-rudiments or potential-rudiments. This characteristic general property of the apical meristem, of forming growth centres (definitely outlined as chemical differentiation), persists during transition to the reproductive state. The problems inherent to this phenomenon are extremely complex. These open new vistas for research and as the work progresses they are likely to illuminate, amplify or qualify some of the earlier views on floral morphology.

Main fields of observations have been usually the dicotyledons—perhaps due to their accessibility in various stages and easy exposure of the apex. Monocotyledons though also investigated to some extent at least pose a problem of the culm differentiation before the flowers could be identified on the axis. Earlier literature has been dealt at length by Barnard (1955, 1961), Murty and Kumar (1970, 1972) etc., taking one or the other aspect of differentiation and morphology of the floral parts.

FACTORS AFFECTING THE TRANSITION

The transition from the vegetative to the reproductive phase of development being probably the most profound ontogenetic change, although has been intensively studied by many physiologists (For recent reviews see Mohr, 1962; Allosop, 1964; Hendrics andBorthwich, 1963; Cutter 1961, 1965 etc.), it is in equal clutches of the anatomists and histologists. However, only little is known about the effects of the various controlling factors at the cellular levlel though a valuable begining along these lines has recently been made. Several authors have pointed out the importance of knowing exactly how those factors, which are important in the induction of flowering like the long night or a light break, affect the apex itself. It is perhaps at this stage that the changes in the shoot growth and development, which are subsequently manifested have their inception.

Recently, Cutter (1965) has reviewed the factors affecting the changes in the state of vegetativeness by which a plant is induced to flower. She is of the opinion that before these changes should occur, the plant must have attained certain size or stage of development-- a condition that is known as "ripeness to flower". There are certainly many evidences in support of this statement. Notably, monocotyledons usually reach a particular age characterstic of the species when they flower and subsequently die. For example, a variety of Bamboo flowers only after a certain stage and strikingly there is no change in this period even under various environmental conditions (Arber, 1925; 1934; Hillman, 1962).

The transition from vegetative to reproductive state for *Triticum aestivum L.* commences when there are apparently 5-7 expanded and externally visible leaves (Barnard, 1955).

The plants sensitive to photoperiods also show an integrated gradual progression towards the flowering state. The question as regards the mechanisms in the shoot apex which control such changes at the time of flowering, remains still unanswered.

Perhaps an endogenous rythm of some kind may be involved in such a change (Hillman, 1962; Cutter, 1965). Murty and Kumar (1970, 1972) also support the theory of "ripeness to flower" in *Fimbristylis diphylla.* It is at certain stage of development only, and not the time in anyway, that the onset of spike is observed. In some cases, as in Sugarcane, this rythm may by delayed (Coleman and Nickell, 1964) in some or the other way though the floral stimulus apparantly persisted for some time. Such a condition may be considered under "intermediate condition" of Gifford and Corson (1971; since the apex in this state usually is neither vegetative nor reproductive). Poux (1957,

1958) observed that the inflorescences of a winter variety of wheat (*T. sativum*) sowed in spring, formed slowly after having about 20 foliage leaves instead of seven. The extra foliage leaves are reported to be due to meristem which even after undergoing transformation to the reproductive state remains cytologically and functionally in an intermediate state. This is confirmed by the form of the apex, disappearance of plastochronic changes and the degree of the activity.

Few organisational and metabolic changes are related to the transition from vegetative to reproductive stages. These changes are well reported and described in many dicotydedons and only in some monocotyledons. Opatrna et al. (1964) report increased stratifications with the time at the apex for *Triticum aestivum*. The zonation pattern is reported to be more discernible. The apex during transition elongates, and at the appearance of the first bract zonation is less clear and gradually disappears when bract and spikelets of the upper half of the ear are initiated. Barnard (1955, 1957a,) studied the floral histogenesis in the representative examples of Graminae. It is reported that the first morphological indication of the spike formation is the rapid elongation of the apical dome. No particular changes in the differentiation are reported.

On the other hand Poux (1960 a. b, c,) has reported that in spring variety of wheat, sown in spring, the apex remained vegetative upto the seven leaf stage, at which time a profound dedifferentiation of the méristème d'attente is purported to occur as revealed by increased staining of RNA. He has therefore, concluded that in the vegetative apex growth is nearly exclusively subapical and essentially apical in the reproductive condition.

Lolium temulentum L. Which responds to an inductive cycle has been intensively studied cytohistologically as regards the floral induction (Knox and Evans, 1966). Throughout the apex RNA concentration is uniformaly high and this condition continued in day II apices, but day III apices show a sign of induction. A large build up of RNA appears at the sites of future spikelet primordia in the axils of bracts. No obvious reduction in nucleohistone staining is reported. These stained equivocally with alkaline fast-green or ammonical silver. The nucleus of the cells of the lower parts in the flank are free of histones, as revealed by bramphol blue staining after deamination or acetylation, but the nuclei in the central core show a little concentration of histones. The nuclei that stain are probably rich in arginine while those which do not, are rich in lysine. Though cytoplasmic staining is reported at all stages of induction, the cytoplasmic histones do not change appreciably. Basing upon these observations several possiblities are suggested but not determined precisely.

However, the views of knox and Evans (1966) are not in accordance with those of Poux. Gifford and Corson (1971) attribute this to a reflection of generic differences or methods employed.

Cytological localization and analyses of polysaccharides, RNA, amino acids, and soluble sugars is reported by Murakami (1960, 1962) in rice plant shoot apices. These metabolites are deposited at the causative event of an organ. The polysaccharide concentration decreases with an increase in sugar, aminoacids, RNA and protein synthesis. These conclusions may also hold true for the reproductive phases of the apex and especially in the case of the flower primordia but the applicability of these conclusions still awaits confirmation.

Certain experimental and analytical observations on these transitional apices are also reported. Evans and Wardlaw (1966) have studied the movement of assimilates and showed that the florigens and the assimilates both may move in the phloem but the floral stimulus is mediated by cytoplasmic streams of sieve tubes or phloem parenchyma. The same course is followed by auxins and gibberallins. Not much work has been done regarding the movement of the metabolites but effect of application of different chemicals, auxins and harmones have been studied. These types of studies are very common in dicotyledons but those in monocotyledons are restricted to the cereals.

The effects of mineral nutrition especially nitrogen, phosphorus and potassium on the structure and physiology of the vegetative and inflorescence apices of rice are studied by Shimizu (1959, 1960 a). The number of tunica layers varies with the concentration of nitrogen. Under high phosphorus feed apex changes its topography and becomes cylindrical with irregular divisions in the corpus. Reverse effect is reported as regards the plastochron functioning which is slow. However, potassium modifies the extreme response to high nitrogen and phosphorus. The highly stratified apex of vegetative phase in controls showed less stratification during transition to flower. Flower differentiation is delayed in high nitrogen feed and is quicker in phosphorus thus effect being quantitative only. In wheat, phosphorus played an important role during transition and for other minerals also the effects are the same as for rice (Shimizu 1960b).

Application of Gibberallic acid results in several cytological and developmental changes. In Barely (Im, 1961, 1962, 1963) application of G A just at the begining of of spikelet differentiation enhances the "heading" by four to five days without any effect on the number of spikelets. Tunica stratifications are decreased due to irregularity of divisional planes in the apex G A application promoted hydrolysis of polysacharides and affected RNA and protein metabolism with increase in the number of mitochondria. Free amino acids concentration also increases in GA treated plants under long day condition. However, nuclear and cytoplasm ratio in the corpus decreases.

Application of acetylene has made pineapple plants to flower as early as three days after treatment (Gifford 1969). The observations reveal nuclear changes and an increase in RNA in the cytoplasm in the cells occupying the apical dome. Nine days after the treatment the apices show marked elongation preceeded by increased activity in the central meristem. Ethylene and applied auxins can also be effective in flowering of pineapples. Evidences are there that the applied auxins bring up more clearly the effect of ethylene by stimulating ethylene formation. Burg and Burg (1966) have made it clear that it is ethylene and not the auxins that make pineapple flower in virto. In this experiment detectable quantities of ethylene are produced after one day of treatment. Sunderland (1961) working with the vernalized seedings of winter wheat has reported that at the time of transition from vegetative to reproductive state, the cells divide once 2.0 days and then there is about a 2·2 fold increase in volume of each succeeding cell generation. In case of non-vernalized seedling the vegetative state is prolonged and the rate of cell division falls off to once every 5.8 days. Tayal et. al. (1961) have reported the day length to affect the size shape and number of primordia at the vegetative apex at an "adult age" ready to flower in two varieties of *Avena sativa.*

Under continuous illumination the apices remained smaller than those kept under 12 hrs. light. However, the former condition hastens the transition to flowering (Tayal et al. 1961).

British *Carex* species studied by Smith (1964) (as quoted by Cutter, 1965) showed a critical condition as regards the sex expression during treatments with IAA, TIBA or kinetin, the future sex organs change their developmetal path as a lateral spike. In Carex *panicea* the treatment of kinetin changed the organisation and formation of spikelets resembling those of a closely related genus *Kobresia* (Smith, 1964)

MORPHOLOGICAL MANIFESTATIONS

Morphological, histological and configurational manifestations have variously been reported in a number of angiosperms but from these studies it is difficult to draw any conclusion as both the possiblities (positive and negative) occur with equal frequency. In many a dicotyledons, no definitive and major change during transition has been reported in plants studied like *Arabiodopsis, Capsella, Anagallis* (Vaughan, 1955); *Oebitgera biennis* (Bersillon, 1957); *Reseda lutea* (Bersillon 1958); *Jussieua grandiflora* (Michaux, 1964) and others. In some other cases, on the other hand lots of changes at histological and configurational levels are reported (see Gifford and Corson, 1971).

In monocotyledons these types of studies are comparatively recent. In Graminae Barnard (1955, 1957 a,) worked out the details of morphological indications of spike formation in one species each of *Lolium, Bronus, Danthonia, Ehrhartia, Bambusa, Stipa* and *Triticum.* It has constantly been observed that the vegetative apex elongates rapidely to a considerable extent before it changes to reproductive state. These observations, however, do not show any marked change in the configuration pattern of the meristems. In *Triticum aestivum* a different pattern of differentiation is observed by Optrna et al. (1964). They stress the zonation becoming more evident with an increase in stratification.

In the vegetative apex of *Avena sativa* L., (Ikenbery, 1966) has reported two layered tunica even from the very beginning of ontogenesis of apex from dormant embryo to nine leaved state. At this stage the plant undergoes transition to flower. The size of the apex is reported to increase along with an increase in the number of cells. The plastochron usually decreases.

Klaus (1966), however, in barley reported the apex to have a single layered tunica (The author uses the Term 'protoderm'. through out the ontogeny). Im (1961, 1962, 1963) observed that the apices treated with Gibberallic acid, when turning to flower show an increased capacity of division in the cells, and that these divisions are irregular and hence there is a decrease in the number of stratifications.

A complete history of ontogenesis in the rice plant is made by Kaufman (1959 a, b, c). The shoot tip including apex and very young leaf primordia, falls under the 'Short type' of Sharman (1942b) As the apex grows older (i.e., prior to the transition and during the transition period) the apex elongates considerably with an increase in the plastchron span. While the single layered tunica (in vegetative condition) appears as two layered in the transitional and floral stages the corpus tends to have stratifications. The problem as to how the second layer of tunica appears in the flowering apex is not explained. Does it appear by splitting of the preexisting one or is it due to the outer most corpus layer appearing as tunica second layer with changed activities and manifestations of the original entity? This type of condition needs a careful attention as it might lead to few more definitive conclusions for the problems facing the present ontogenetic determination of homologies.

However, Kaufman's observations are contrary to the earlier views regarding the fluctuations in the size of the apex during vegetative as well as floral plastochron. The tunica stratifications are reported to vary according to the order of branching as well. A discrete tunica is less stable in primary and secondary branches while spikelet apices have usually one layered tunica.

Zamora (1959), also working on rice plant, but of another variety viz., Elon-elon confirmed the vegetative apex to have a biseriate tunica and a clear zonation pattern in the corpus. However, the size of the apex is reported to be much greater than that studied for the variety Caloro by Kaufman. Cavendish banana (Musaceae) is extremely interesting and has been extensively studied by two schools. Barker and Steward (1962a, b) reported that the flowering apex develops from the axillary bud which is initially vegetative. During transition, the apex enlarges in size greatly. They reported very little demarcation of different zones in the vegetative apex. There is only one sharply defined surface layer and the second is detectable in various developmental stages. Fahn et. al. (1963), of the other school, are more insistent about the zonation of the apex. They reported that the zonation is more clear, and more particularly, the central mother cell zone below the tunica is found to be in an active stage. Almost similar condition is reported by one of us (Kumar 1971) in *Musa coccinea*. In this species, apart from single layered tunica, the number of the stratifications do range during the various stages of a plastochron. The central mother cell zone is shown to be very active and is seen to divide in various planes during mid area phase. The cambium like zone (See. Papham and Chan 1950 and 1952), also manifests for a short time during mid to early maximal area phase. Fahn et al. (1963) also reported this zone but Gifford and Corson (1971) doubt this report.

Barker and Steward (1962a) observe that the lateral buds which gave rise to actual flowering apices are not derived from the actual apical meristem and are separated from it in time and by space of ten interposed leaves. The vegetative shoot tip which is a broad flattened dome in a central depresssion increses considerably in height and is cone like during the transition period.

Both the schools, however, are in general agreement about the number of stratifications during flowering. There are at least 3 stratifications which very upto eight. The cells of this zone divide actively and the activity is correlated with the rib meristem as well. Ram et al. (1962) also have studied the changes during transition to flowering and have reported the flowers of a hand to develop simultaneously.

Studies on *Triticum sativum* (Poux, 1957, 1958) and *T. valgare* (Poux, 1960 a, b, c) a profound differentiation is reported after the seventh leaf stage. The apex when turning to flower starts the apical growth whereas during the vegetative phase it is mainly subapical.

In rye the mitotic activity of the apex increases when it is about to flower (Sunderland, 1961) and also, there is an appreciable change in the number and total volume of the cells produced, 2.2 fold increase in the volume of each succeeding cell generation during transition and 2.4 fold increase after transition in the volume of succeeding cell generation is observed. The cells also show an increased divisional capacity and divide once every 1.4 days.

Knox and Evans (1966) on the basis of cytohistological evidences conclude that there is no evidence for méristéme d' attente at the main vegetative apex in *Lolium temulentum.* They compare the inactive but potentially responsive axillary bud sites to the central zone of dicotyledons.

Murty and Kumar (1972) studying the floral histogenesis in *Fimbristylis diphylla* report that from the vegetative to reproductive state, apex although does not show any variation in the number of tunica layers, the corpus shows less demarcated zonation pattern. The apex, however, elongates to a considerable extent and then divides into several spikelet primordia by cessation of growth at certain points. These spikelet primordial apices also have the same configuration as at the time of differentiation and transition. At the base of the main axis of the culm and that of each spikelet, intercalary meristems are reported. The manifestations of the flowering impulse, which moves spirally through the apex, are in the form of highly dense cytoplasmic cells at the points of impulse. The flower primordia are reported to originate when the impulse or rythm has passed through the site. These authors on the basis of cytological evidences and the divisional capacity of the cells have denied the presence of méristéme d' attente.

Plantefol (1946, 1947) and his associates during the studies of considerable number of species of vascular plants affirmed the presence of an 'anneau initial'. They also asserted that the central terminal cells of the axis have no histogenic significance during vegetative growth and become active only in the formation of the terminal flower or inflorescence (Buvat, 1952a,b, 1953, 1955, 1958; Camfort, 1956a,b; Catesson 1953, Lance, 1952, 1954 a,b, 1957). In cases where the terminal flower does not exist this inactive zone is called the "Zone apicale or Zone exiale". Several books and reviews appraise the earlier works (Clowes, 1961: Cutter, 1959; Esau, 1965; Gifford, 1954; Gifford and Wetmore, 1957; Lance, 1957; Wardlaw, 1956, 1957, and 1965b), and lot of criticism has arisen as regards the presence and differentiation of this initiating zone. Buvat and Geneves (1951), Buvat and Liard (1953) and Plantefol (1962) on the basis of cytology of this particular region reemphasize their contention, of course, with some consideration to few divisions observed in the vegetative phase.

Brooks (1940) from the study of the vegetative and floral apices of *Amygdalus communis* supported Grégoire's contention. On the other hand this contention has been refuted by Satina and Blakeslee (1941, 1943). These authors working with the periclinal chimaras of *Datura stramonium* L. found no essential differences between the histogenesis of apical meristems in vegetative and flowering state.

In *Frasera carolinensis walt.* (McCoy, 1940), *Rubus spp.* (Engard, 1944), *Vinca rosea* (Boke, 1947, 1949) it has been reported that the foliage leaves and all the floral organs arise in the same manner, therefore, have subscribed entirely to the classical concept.

The histological data on *Triticum aestivum* (Barnard, 1955) during histogenesis are somehow in general agreement to the previous observations of Percival (1921) and Bonnett (1936) with slight modifications. After 7 clearly visible leaves the apex (vegetative) undergoes transition to the reproductive stage with a morphological manifestation as an elongated cone. At this time several leaf initials are formed in close succession up to 14th plastochrons. The 10th leaf primordium is large enough at this time to overtop the dome shaped apex. The author (Barnard, 1955) called this leaf as 'Cowl'. The fourteenth leaf is however only a crescent shaped ridge of tissue. The elongation of apical dome continues till there are about 16 leaf initials in between the 'cowl' and the exact dome.

The flower primordium is reported (Barnard, 1955) to develop essentially in the same manner as that of the axillary vegetative bud and spiklet in wheat. The organisation of this region is also claimed to be same as in the vegetative axis. Same condition is reported in other studies on Juncaceae (Barnard, 1958).

It can be concluded in the last, however, that the process of differentiation of the vegetative apex into the flowering apex is a complex phenomenon, perhaps controlled by several factors. These factors collectively affect the transition. The main problem as to how these factors affect firstly at the cellular level and secondly at the cell aggregates as an entity level alongwith internal interactions still remains to be solved, thus a composite problem which should be worked out in detail by collaborations amongst morphologists, morphogenesists, physiologists and the geneticists. Somehow, the fact cannot be overlooked and overemphasized that a complete knowledge of developmental anatomy will allow the geneticist and physiologist to view the component parts of this complex problem, which will, then enable them to design more precise experiments and draw more accurate inferences from the data thus obtained.

FUTURE PROSPECTS

Many evidences now exist today to confirm that the differentiation from the vegetative state to flowering state is due to some metabolites moving into the apex. This is of course a very interesting and profitable field of experimentation and there is hardly any doubt that in not very distant future, much more will have been discovered about the nature, path, and the synthesis of the particular harmones responsible for the flowering, and more so, in case of different floral organs.

Application of various chemical substances either synthesized or otherwise known to occur naturally in plant tissues can still give us more important information by producing many modifications of growth and development. Although the observed modifications are difficult to interpret in metabolic terms because of firstly failure to observe effects on very early stages of growth and secondly failure to distinguish clearly between effects on inception and subsequent development of the organ; however, they give us an impetus to observe carefully the changing components of the apex and thus may unfold to some extent, the mystery of differentiation. Then, the establishment of growth centres in the floral apex is a localized modification within the vegetative apex, perhaps controlled primarily by sequential evocation of genes as suggested by Wardlaw (1968) and secondarily by the metabolites moving into the apex. Thus its formation is stimulated, regulated and controlled by factors extrinsic or intrinsic. To work out these factors and their interactions within, may in future, bring out more information on differentiation.

References

Allosop, A. 1964. Shoot morphogenesis. *Ann. Rev. Pl. Physiol.* **15:** 225-254.

Arber, A. 1925. *Monocotyledons. A morphological Study.* Cambridge University Press, Cambridge.

——. 1934. *The Graminae. A study of Cereal, Bamboo and Grass.* Cambridge University, Press, Cambridge.

——. 1937. The interpretation of the flower : a study of some aspects of morphological thought. *Biol. Rev.* **12:** 157-184.

Bancroft, H. 1935. A review of researches concerning floral morphology. *Bot. Rev.* **1:** 77-99.

Barker, W. G., and F. C. Steward. 1962a. Growth and development of the banana plant 1. The growing regions of the vegetative shoot. 2. The transition from the vegetative to the floral shoot in *Musa acuminata* CV. Gros Michel. *Ann. Bot N.S.* **26:** 389-411.

——, and——. 1962b. Growth and development of the banana plant. 3. The transition from the vegetative to the floral shoot in *Musa acuminata* CV. Gros Michel. *Ann. Bot. N.S.* **26:** 413-423.

Barnard, C. 1955. Histogenesis of the inflorescence and flower of *Triticum aestivum L. Aust. J. Bot.* **3:** 1-20.

——. 1957a. Floral histogenesis in the monocotyledons. I. The Gramineae. *Aust. J. Bot.* **5:** 1-20.

——. 1957b. Floral histogenesis in the monocotyledons. II. The Cyperaceae. *Aust. J. Bot.* **5:** 115-128.

——. 1958. Floral histogenesis in the monocotyledons. III. The Juncaceae. *Aust. J. Bot.* **6:** 285-298.

——. 1960. Floral histogenesis in the monocotyledons. IV. The Liliaceae. *Aust. J. Bot.* **8:** 213-225.

——. 1961. The interpretation of the angiosperm flower. *Aust. J. Sci* **24:** 64-92.

Bersillon, G. 1957. Ontogénie comparée de la tige feuillée et de l'inflorescence chez *Oenothera biennis* L. *C.r. Acad. des Sci., Paris* **245:** 1455-1458.

——. 1958. L'inflorescence terminal de *Reseda lutea L.,* Ses rapports avec la pousse végétative. *Rev. de Cytol. et. Biol. Vég.* **19:** 185-197.

Boke, N.H. 1947. Development and adult shoot apex and floral initiation in *Vinca rosea* L. *Am. J. Bot.* **34:** 433-439

——. 1949. Development of stamens and carpels in *Vinca rosea. Am. J. Bot.* **36:** 535-547.

Bonnett, O. T 1936. Development of wheat spike. *J. Agric. Res.* **53:** 445-457.

Brooks, R.M. 1940. Comparative histogenesis of vegetative and floral apices in *Amygdalus communis,* with special reference to the carpel. *Hilgardia.* **13:** 249-306.

Burg, S. P., and E. A. Burg. 1966. Auxin-induced ethylene formation: its relation to flowering in the pineapple *Science* **152:** 1269.

Buvat. R. 1952a. Structure evolution et fonctionnement du méristème apical de quelques dicotylédones. *Ann. Sci. Natl. Bot. Ser.* **XI. 13:** 199-300.

——. 1952b. L'organisation des méristèmes aplicaux chez les végétatix vasculaires. *Union des Net. Bull.* **40:** 54-66.

——. 1953. L'apex de *Triticum vulgare:* modalites de reprise de mitoses lors de la germination et du fonctionnement végétatif. *C.r. Acad. Sci., Paris* **236:** 1989-1991.

——. 1955. Le méristème apical de la tige. *Ann. Biol.* **31:** 596-656.

——. 1958. Recherches sur les infrastructures due cytoplasme dans les cellules du méristeme apical, des ébauches foliaires et des feuilles développées d' *Elodea canadensis. Ann. Sci. Natl. Bot. Ser.* II. **19:** 121-158

——, and L. Geneves. 1951. Sur l'inexistence des initiales axiales dans la rans la racine d' *Allium cepa* L. (Liliacees). *C.r. Acad. Sci., Paris* **232:** 1579-1581

——, and O. Liard. 1953. Nouvelle constation de l'inerie des soi-disants initiales axoalesdens le méristème radiculaire de *Triticum vulgare. C.r. Acad. Sci., Paris* **236:** 1193–1195.

Camefort, H. 1956a Etude de la structure du point vegetatif et des variations phyllotaxiques chez quelques Gymnospospermes *Ann. Sci. Natl., Bot. Ser* XI, **17:** 1-185.

——. 1956b. Structure de l' apex caulinaire des gymnosperme es. *Ann. Biol.* **60:** 401–416.

Catesson, A. M. 1953. Structure évolution et fonctionnement du point végétatif d'unte Moncotylédone: *Luzula pedemontana* Boiss. et Reut (Joncacees). *Ann. Sci. Natl. Bot. Ser* XI. **14:** 253-291.

Clowes, F. A. L. 1961. *Apical Meristems.* Blackwell, Oxford.

Coleman, R. E., and L. G. Nickel. 1964. Stability of flowering stimulus in isolated stem tips of sugarcane. *Nature, Lond.* **201:** 941-942.

Cutter, E. G. 1959. On a theory of phyllotaxis and histogenesis. *Biol. Rev.* **34:** 243–263.

——. 1961. The inception and distribution of flowers in the Nymphaeaceae. *Proc. Linn. Soc. (London)* **172:** 93–100

——. 1965. Recent experimental studies of the shoot apex and shoot morphogenesis. *Bot. Rev.* **31:** 7–113.

——. 1971. *Plant Anatomy* II *The Organs.* Arnold, Lond.

Douglas, G. E. 1957. The inferior ovary. *Bot. Rev.*, **23:** 1–46.

Eames, A. J. 1961. *Morphology of Angiosperms.* McGraw Hill. N.Y.

Eckert, G. 1966. Entwicklungsgeschichtliche and Blutenanatomische Untersuchungen Zur Problem der Obdiplostemonie. *Bot. Jahr.* **85:** 523–604.

Engard, G. J. 1944. Organogenesis in *Rubus. Res. Publ. Univ. Hawaii.* **21:** I.

Esau, K. 1965. *Plant Anatomy* (Second Edition). John Wiley. New York.

Evans, L.T., and C. W. Wardlaw. 1966 Independent translocation of 14C–labelled assimilates and of the floral stimulus in *Lolium temulentum. Planta* **68:** 310–326

Fahn, A., S. Stoler, and T. First. 1963. Vegetative shoot apex in banana and zonal changes as it becomes reproductive. *Bot. Gaz.* **124:** 246–250.

Gifford, E. M. Jr. 1954. The shoot apex in angiosperms. *Bot. Rev.* **20:** 477–529.

——. 1969. Initiation and early development of inflorescences in pineapple (*Ananas comosus* Smooth-Cayenne) treated with acetylene. *Am. J. Bot.* **56:** 892–897.

——, and G. E. Corson. 1971. The shoot apex in seed plants. *Bot. Nev.* **37:** 143–229

——, and R. H. Wetmore. 1957. Apical meristems of vegetative shoots and strobili in certain gymonsperms *Proc. Natl. Acad. Sci.* (*U.S.*) **43:** 561–577

Goethe, J.W. 1790. *Versuch die Metomorphose der Pflanzen zu erklären.* Gotha.

Gregoire, V. 1938. La morphogenése et l'autonomie morphologique de l'appareil floral, I: Pe carpelle. *Cellule* **47:** 285.

Heel, W.A. Van. 1966. Morphology of the androecium in Malvales. *Blumea* **13:** 177–394.

——. 1969. The Synangial nature of pollen sac on the strength of "Congenital fusion and conservatism of the vascular bundle system" With special refernce to some Malvales *Kon. Nederl. Akad. Wetensch. Proc.* **72:** 172–206.

Hendricks, S. B., and H. A. Borthwick. 1963. Control of plant growth by light. In *Environmental Control of Plant Growth.* L.T. Evans (ed.). Academic Press, New York and London. 233–263.

Heslop-Harrison, J. 1964. Sex expression in flowering plants. In *Meristems and Differentiation.* Brookhaven Symposia in Biology. **16:** 109-125.

Hillman, W. S. 1962. *The Physiology of Flowering.* Holt. Rinehart and Winston. N. Y.

Ikenberry, G. J. Jr. 1966. Reactivation of the dormant embryo, and initiation and development of buds and leaves on *Avena sativa. Iowa State Jour. Sci.* **41:** 7–23.

Im, H. B. 1961. The effect of gibberellin on the differentation and development of barley spikes. *Seoul Univ. Jour.* **10D:** 55–67.

——. 1962. The effect of gibberellin on the leaf development and the cytohistological zone of shoot apices in barley plants. *Seoul Univ. Jour.* **12D:** 25–34.

——. 1963. Study of gibberellin effects on the differentiation and development of barley spikes. *Seoul Univ. Jour.* **14B:** 47–92.

Jones, H. A., and S. L. Emsweller. 1936. Development of the flower and marogametophyte of *Allium cepa. Hilgardia* **10:** 415-428.

Kaufman, P. B. 1959a. Development of the shoot of *Oryza sativa* L.I. The shoot apex. *Phytomorphology* **9:** 228–242.

——. 1959b. Development of the shoot of *Oryza sativa* L. II. Leaf Histogenesis. *Phytomorphology* **9:** 277–311.

——. 1959c. Development of the shoot of *Oryza sativa* L.III. Early stages in histogenesis of the stem and ontogeny of the adventitious root. *Phytomorhology* **9:** 382–404.

Klaus, H. 1966. Ontogenetische and histogenetische Untersuchungen an der Gerste (*Hordeum distichon* L.) *Bot. Jahrb.* **85:** 45-79.

Knox, R. B., and L. T. Evans. 1966. Inflorescence initiation in *Lolium temulentum* L. VIII. Histochemical changes at the shoot apex during induction. *Aust. J. Biol. Sci.* **19:** 233–245.

Kumar, V. 1971. *Apical Meristems and Development of Lateral Organs in Zingiberales.* Ph. D. Thesis, Meerut University, Meerut.

Lance, A. 1952. Sur la structure et le fonctionnement du point vegetatif de *Vicia faba* L. *Ann. Sci. Natl. Bot. Ser.* XI. **13:** 301–339.

——. 1954a. Evolution histologique de l'apex d' *Aster sinensis. C.r. Acad. Sci., Paris* **238:** 1442–1444.

——. 1954b. Transformation du point vegetatif d' *Aster sinensis* en meristeme d'inflorescence. *C.r. Acad. Sci., Paris* **238:** 2437–2439.

——. 1957. Recherches cytologiques sur l'evolution de quelques meristemes apicaux et sur ses variations provoquees per des traitements photoperiodiques. *Ann. Sci. Natl. Bot. Ser.* XI. **18:** 91–422.

McCoy, R. W. 1940. Floralor ganogenesis in *Frasera carolinensis Am. J. Bot.* **27:** 600–609.

Melville, R. 1962. A new theory of the Angiosperm flower. I The Gynoeciom. *Kew Bull.* **16:** 1–50.

——. 1963. A new theory of the Angiosperm flower. II The Androecium. *Kew Bull.* **17:** 1–63.

Michaux, N. 1964. Structure et evolution du meristeme apical, du *Jussieua grandiflora* Michx., durant les phases vegetative et reproductirice. *Rev. Gen. Bot.* **71:** 91–170.

Mohr, H. 1962. Primary effects of light on growth. *Ann. Rev. Pl. Physiol.* **13:** 465–488.

Murakami, K. 1960. Studies on morphological and physiological changes in shoot apex of rice plant during floral differentiation I. Distribution of polysaccharides in observed under short-day treatment. *Jap. J. Breeding* **10:** 75–80. (With English Summary).

——. 1962. Studies on morphological and

physiological changes in shoot apex of rice plant during floral differentiation. II. Changes in quality of RNA. protein, amino acids and soluble sugar. *Jap. Jour. Breeding* **12:** 85-92 (With English Summary)

MURTY, Y. S., AND V. KUMAR. 1970. Floral histogenesis in *Fimbristylis diphylla* Vahl. Abs. *Proc. Indian Sci. Congr. 54th session Kharagpur.*

——, AND——. 1972. Flowering impulse and floral histogenesis in *Fimbristylis diphylla* Vahl. (In Press)

OPTRNA, J., F. SEIDLOVA, AND K. BENES. 1964. The anatomy of the shoot. apex of wheat (*Triticum aestivum* L.) during transition from the vegetative to the reproductive state and the determination of the primordia. *Biol. Plant. Acad. Sci. Bohemoslov.* **6:** 219–225.

PAYER, J. B. 1957. *Traite d'organogenie Comparie de la Fleur.* Paris, Librarie de Victor Masson.

PERCIVAL, J. 1921. *The Wheat Plant.* Duck work and Co. London.

PHILIPSON, W. R. 1946. Studies in the development of the inflorescence. I. The capitulum of *Bellis perinnis* L. *Ann. Bot. N.S.* **10:** 257.

——. 1947a. Studies in the development of the inflorescence. II. The capitula of *Succisa pratensis* Moench. and *Dipsacus fullomum* L. *Ann. Bot., N.S.* **11:** 285.

——.1947b. Studies in the development of the inflorescence. III. The thyrse of *Valeriana officinalis* L. *Ann. Bot. N.S.* **11:** 409.

——. 1948a. Studies in the develoment of the inflorescence. IV. The capitula of *Hieracium boreale* Fries and *Dehlia gracilis* Ortg. *Ann. Bot. N.S.* **12:** 65.

——. 1948b. Studies in the develpment of the inflorescence. V. The raceme of *Lobelia dortmann* L. and other Campanulaceous inflorescences. *Ann. Bot. N.S.* **12:** 147–156.

——. 1949. The ontogeny of the shoot apex in dicotyledons. *Biol. Rev.* **24:** 21–50.

PLANTEFOL L. 1946. Fondements d'une theorie phyllotaxique novuelle I Historique et critique. II. La phyllotaxie des monocotyledones. *Ann. Sci. Nat. Bot.* XI **7:** 153–229.

——. 1947. Fondements d'une theorie phyllotaxique novuelle III. La phyllotaxi des dicotyledones IV. Generalisations et conclusions. *Ann. Sci. Natl. Bot. Ser.* XI. **8: 1-71.**

——. 1962. Structure et fonctionnement du meristeme terminal de la tige. Rapport introductif. *Mim. Sci. Bot., France* **1962:** 3–14.

OPHAM R.A., AND A.P. CHAN. 1950. Zonation in the vegetative stem tip of *Chrysanthemum morifolium* Bailey. *Am. J. Bot.* **37:** 476–484.

——, AND ——. 1952. Origin and development of the receptacle of *Chrysanthemum morifolium. Am. J. Bot.* **39:** 329– 332.

POUX, N. 1957. Comportement du meristeme apical d'un Ble d'hiver (*Triticum satvium* Lamk.) seme au printemps. *C.r. Acad. Sci., Paris* **245:** 2522–2525.

——. 1958. Comportament du meristeme apical de deux varietes de Ble, *Triticum sativum* Lamk., L'une de printemps, l'autre d'automne, semees simultanement au printemps. *Rev. de Cytol. et Biol. Veg.* **19:** 273–305.

——. 1960a. Variations de la repartition des ribonucleoproteins dans l'apex d'un Ble de printemps (*Triticum vulgare* Vill.) au cours du development. *C.r. Acad. Sci., Paris.* **250:** 585–587.

——. 1960b. Evolution cytologique du meristeme apical d'un Ble de printemps. (*Triticum vulgare Vill.*, var. *Vilmorin* Werna). *C.r. Acad. Sci., Paris* **250:** 1099–1101.

——. 1960c. Variations de la repartition des ribonucleoproteines dais les apex d'un Ble d'hiver (*Triticum vulgare* Vill.) same, soit au printemps, solt en automne. *C.r. Acad. Sci., Paris.* **251:** 1904–1906.

PURI, V. 1951. The role of floral anatomy in the solution of morphological problems. *Bot. Rev.* **17:** 471–553.

——. 1952. Placentation in Angiosperms. *Bot. Rev.* **18:** 603–651.

——. 1964. On the relation between ovule and carpel. *J. Indian bot. Soc.* Maheshwari Commemoration Volume **42A:** 189–198.

RAM, H.Y. M., M. RAM, AND F. C. STEWARD. 1962. Growth and development of the banana plant. III. A. The origin of the inflorescence and the development of the flowers. B. The structure and development of the fruit. *Ann. Bot. N.S.* **26:** 657–637.

SATINA, S, AND A. F. BLAKESLEE. 1941. Periclinal chimeras in *Datura stramonium* in relation to the development of leaf and flower. *Am. J. Bot.* **28:** 862–871.

——, AND ——. 1943. Periclinal chimeras in *Datura* in relation to the development of the carpel. *Am. J. Bot.* **30:** 453–462.

SHARMAN, B. C. 1942a. Developmental anatomy of the shoot of *Zea mays. Ann. Bot. N.S.* **6:** 245–282.

——. 1942b. Shoot apex in grasses and cereals. *Nature, Lond.* **149:** 82–83.

——. 1947. The biology and developmental morphology of the shoot apex in the Gramineae. *New Phytol.* **46:** 20–34.

SHIMIZU, M. 1959. Effects of nitrogen, phosphorus and potassium on the structure and physiology of the vegetative shoot apex in the rice plant. *Jap. J. Breeding* **9:** 79–88. (with English summary).

——. 1960a. Effects of nitrogen, phosphorus and potassium on the panicle differentiation and the time of heading in rice plants. *Jap. Jour. Breeding* **10:** 52–60. (with English summary).

——. 1960b. Effects of nitrogen phosphorus and potassium on the spike differentiation and development in wheat plants. *Jap. Jour. Breeding* **10:** 179–187 (with English summary).

SMITH, D. L. 1946. The inflorescence of *Carex.* (unpublished quoted by Cutter, 1965).

STERLING, C. 1963. The affinities of *Prinsepia* (Rosaceae). *Am. J. Bot.* **50:** 693–9.

SUNDERLAND, N. 1961. Cell division and expansion in the growth of the shoot apex. *J. Exptl. Bot.* **12:** 446–457.

TAYAL, M. S., M.P. KAUSHIK, AND K.K. NANDA. 1961. Growth and phasic development of oat. I. A developmental study of the shoot apex in two varieties of *Avena sativa* under differential photoperiodic treatments. *Indian Jour. Plant Physiol.* **4:** 132–149.

VAUGHAN, J. G. 1955. The morphology and growth of the vegetative and reproductive apex of *Arabidopsis thaliana* (L.) Heynh., *Capsella bursa pastoris* (L.) Medic. and *Anagallis arvensis* L. *J. Linn. Soc. Bot.* **55:** 279–301.

WARDLAW. C. W. 1956. Generalization on the apical meristem. *Nature, Lond.* **178:** 1427–1429.

—— 1957. The floral meristem as a reaction system .*Proc. Roy. Soc. Edinb. B.* **66:** 394-408.

——. 1961. The inception of shoot organization. In *Recent Advances in Botany*. Univ. Toronto Press. 816–820.

——. 1964. Gibberellic acid and flowering in *Petasites hybridus. Nature Lond.* **202:** 575–577.

——. 1965b. The organization of the shoot apex. *Encyc. Pl. Physiol.* **15:** 966–1076.

——. 1965b. *Organization and Evolution in Plants.* Longmans Green and Co., London.

——. 1968. *Morphogenesis in Plants: A Contemporary Study.* Methuen and Co., London.

WILSON, C. L., AND T. JUST 1939. The morphology of the flower. *Bot. Rev.* **5:** 97–131.

ZAMORA, P.M. 1959. Zonal structure of the apex of *Oryza sativa* L. *Philippine Agr.* **42:** 391–403.

Adv. Pl. Morph. 1972: 304–314

ETHEPHON AND PLANT DEVELOPMENT : AN APPRAISAL

By H. Y. Mohan Ram and V. S. Jaiswal
Department of Botany, University of Delhi, Delhi–7, India

Abstract

Recent evidence suggests that ethylene should be considered as a plant hormone. Being a gas, produced in very small quantities by plant tissues, its detection and role in physiological processes had to await refined techniques like gas chromatography.

The synthesis and availability of ethephon (2-chloroethylphosphonic acid), a liquid, that breaks down in plant tissues to release ethylene, has enabled the physiologists to conduct numerous experiments for understanding the mechanism of action of ethylene. Because of the ease with which ethephon can be handled, numerous uses to which it can be applied in plant production have come to prominence in the past three years.

Ethephon brings about profound changes in Plant development. If stimulates seed germination and sprouting of dormant corms and tubers, inhibits terminal shoot growth, enhances lateral branching, increases resistance to lodging and promotes grain yield. It accelerates leaf abscission, initiates rooting and stimulates latex flow in rubber trees. The ability of ethephon to induce flowering in pineapple and control flowering and biennial bearing in mango holds much promise for future. Ethephon also induces flowering in short-day plants like *Plumbago indica* and *Utricularia inflexa* under non-inductive conditions.

Treatment with ethephon causes the production of female flowers on male plants of *Cannabis sativa* and either delays male flower formation or totally suppresses it in the cucurbits. In wheat ethephon induces male sterility.

In fruit crops, ethephon application helps in planning precise harvest timings, concentrating fruit maturity, colour and quality, and facilitating mechanical or hand harvesting by reducing fruit removal force.

The mode of action of ethephon is not independent of that of ethylene. The way in which ethylene acts is by no means understood. It is believed that it binds itself with molecules like proteins and modifies their structure and activity.

As with other hormones, knowledge about the physiological effects of ethylene is accumulating at a much faster rate than our understanding of the nature of its action.

Introduction

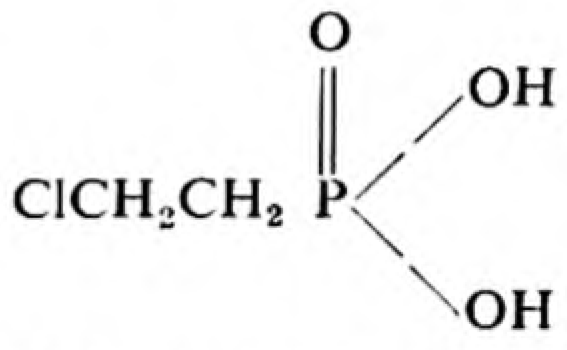
As early as the middle of the last century, farmers had noted several remarkable plant growth responses caused by illuminating gas and smoke. In 1901 D. Neljubow showed that ethylene (C_2H_4) was a component of these sources and was principally responsible for the effects observed. However, it was the historical experiment of Zimmerman and Wilcoxon (1935) which demonstrated that auxins and ethylene evoked similar responses that led to the suggestion that ethylene may be a plant hormone (Crocker et al., 1935). Improvement in the technique of estimating very low levels of ethylene, especially by gas chromatography, has provided evidence that ethylene is a natural plant product. and is associated with certain physiological phenomena (Pratt and Goeschl, 1969).

The only two gases which had been known to participate in the metabolism of plants were oxygen and carbon dioxide. The evidence that ethylene is a physiologically important gas led many plant physiologists to investigate its role in plant growth and development. Being a gas, ethylene is very inconvenient to handle and expensive to use, particularly under field conditions. It was fortuitous that a new synthetic compound bearing the name of ethephon showed the unique property of releasing ethylenein plant tissues (Amchem Technical data sheet, 1967). This compound when applied as a foliar spray invokes all the ethylene-induced responses in plants and has proved to be invaluable in understanding the hormonal role of ethylene.

In this article we wish to present an up-to-date but brief review of the nature of the compound, its various developmental effects, the probable mode of action and its application in commercial plant production.

Chemical Nature and Properties

Ethephon (2-chloroethylphosphonic acid) was introduced as a plant growth regulator in 1967 by Amchem Products Inc. Ambler, Pennsylvania, U. S. A. The compound has been known by several trade names : ACP 68-64, Amchem 66-329, CEPA, Ethrel and 2-chloroethanephosphonic acid.



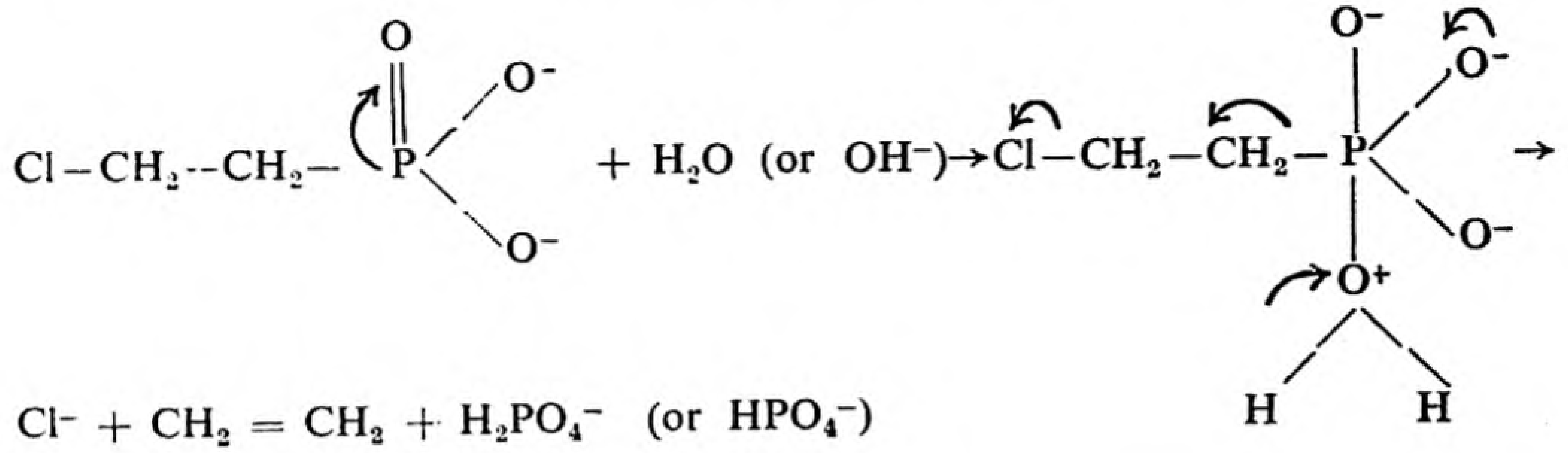
2-chloroethylphosphonic acid

The acid is a hygroscopic crystalline substance, highly soluble in water and other polar solvents, with a molecular weight of 144.5. It is stable in aqueous solution below pH 3.5 and disintegrates as the pH rises above 4. The technical substance is available in three formulations (which have a pH of about 1): (1) acid, ester and anhydride; (2) free acid (up to 90%); (3) ester.

Ethephon is relatively non-toxic, but becuase of its strong acidic nature it may be irritating to exposed skin and eyes.

Two Russian chemists, Kabachnik and Rossiiskaya (1946) reported the synthesis of 2-chloroethanephosphonic acid by reacting ethylene oxide with phosphorus trichloride. The ethylene liberating properties of this acid were reported by the Australian chemists, Maynard and Swan in 1963.

The chemical mechanism of ethylene production from the compound suggested by Maynard and Swan involves the nucleophilic attack on the phosphonate dianion by a water molecule and the concerted elimination of chlorine, leading to direct formation of phosphate and chloride.

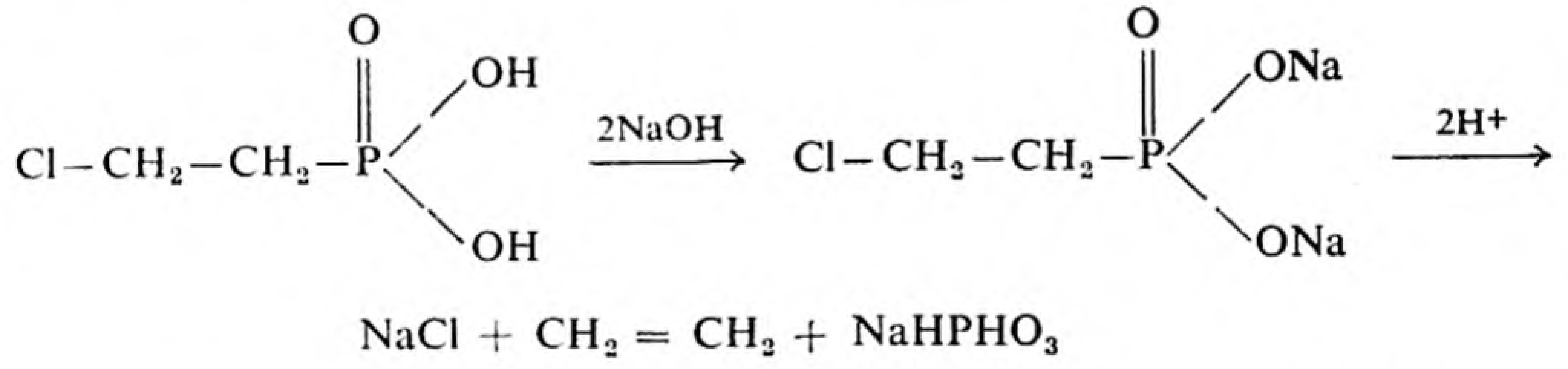


$$\mathrm{Cl{-}CH_2{-}CH_2{-}P(=O)(O^-)_2} + \mathrm{H_2O}\ (\text{or } \mathrm{OH^-}) \rightarrow \mathrm{Cl{-}CH_2{-}CH_2{-}P(O^-)_3(O^+H_2)} \rightarrow$$

$$\mathrm{Cl^-} + \mathrm{CH_2 = CH_2} + \mathrm{H_2PO_4^-}\ (\text{or } \mathrm{HPO_4^-})$$

Probably the OH ion also serves as a nucleophile in the reaction.

Warner and Leopold (1969) studied ethylene evolution from ethephon and proposed a reaction mechanism which involves the removal of phosphonate as phosphonate salt, followed by dehydrohalogenation.



$$\mathrm{Cl{-}CH_2{-}CH_2{-}P(=O)(OH)_2} \xrightarrow{\mathrm{2NaOH}} \mathrm{Cl{-}CH_2{-}CH_2{-}P(=O)(ONa)_2} \xrightarrow{\mathrm{2H^+}}$$

$$\mathrm{NaCl + CH_2 = CH_2 + NaHPHO_3}$$

PHYSIOLOGICAL EFFECTS AND APPLICATIONS

Seed germination, growth of seedlings and bud dormancy. — Freshly harvested seeds of strawberry remain dormant for a long period. Germination is extremely slow and several months are required to complete it. Treatment of freshly harvested seed with 1000, 2500 and 5000 ppm of ethephon for 24 hours induced up to 90% germination in 4 weeks, whereas untreated seeds showed only 20% germination during the same period (Iyer et al., 1970). Virginia type groundnut seeds from apical and basal stem location show "primary" inherent dormancy at harvest (Ketring and Morgan, 1970). Dormancy was overcome by soaking the seeds for 16 hrs in a 10^{-3} M or 5×10^{-4} M aqueous solution of ethephon. Soaking of cotton seeds (free of lint fibres) in ethephon has also shown enhanced percentage of germination (Amchem Technical data sheet, 1970). Seeds of witchweed (*Striga lutea*) require an association with host root or its exudate for effective germination. Ethephon has stimulated the germination of dormant witchweed seed placed in 1 to 1000 ppm solution or in non-sterilized eustris loamy sand containing ethephon at 10 and 100 mg/kg of soil (Egley and Dale, 1970). Treatment of apple fruits with ethephon one month before optimum maturity, promoted seed germination considerably (Coston, 1971).

Ethephon stimulates the sprouting of dormant rhizomes and stolons. This pretreatment is useful in the eradication of stubborn perennating weeds by herbicides. Dip treatments of corms of gladioli stored at high temperature or for a short period at 5°C enhanced sprouting, corm splitting, increase in the number of flower spikes and delay in flowering. The increased vigour and greater survival of corms were of much applied value (Halevy et al., 1970).

Ethephon caused retardation of elongation of root, hypocotyl and epicotyl of *Phaseolus radiatus* seedlings and decreased the number of laterals (Sankhla and Sankhla, 1970). The hypocotyls of treated seedlings also showed coiling.

Thomas and Benedict (1969) found that ethephon treatment inhibited only the tall (Alaska) pea when applied either during seed imbibition or as post-emergence spray. In the dark, however, both tall and dwarf (Progress No. 9 and Early June) varieties were inhibited, the tall plants showing greater inhibition. Soaking of Alaska (tall) and Early June (dwarf) variety pea seeds in ethephon delayed emergence and decreased survival in the former but increased the survival in the latter between 14 and 18°C (Benedict and Thomas, 1969).

Influence on vegetative growth.—One of the significant effects of ethephon is inhibition of terminal growth of shoots and stimulation of lateral branching, which could be profitably employed for induction and delay in flowering, maturation of fruit and increasing plant density. Ethephon treatment of cereals like spring wheat and corn at the tillering stage induced the same effects as those of CCC—reduction in height, increase in the stiffness of straw and resistance to lodging and enhanced grain yield (Karchi, 1969).

In grape vines, ethephon application has been shown to replace the practice of pruning and stimulate uniform maturity of fruit clusters (Weaver and Pool, 1969).

Larsen (1970) has shown that repeated applications of ethephon (250 to 2500 ppm) are effective in defoliation of nursery stocks of apples, Bartlett pears, Italian prune, roses and flowering trees. Whereas urea, potassium iodide and bromodine enhance defoliation when combined with ethephon, spraying with NAA (5 or 10 ppm) 2 weeks before ethephon application significantly decreased the effects of the latter. Under field conditions, defoliation of cotton plants prior to machine harvesting of the bolls has been found to be stimulated by ethephon. Under experimental conditions ethephon promotes abscission of intact, young and old green leaves, petiole explants and flower buds of *Gossypium hirsutum*. Abscission is preceeded by evolution of ethylene. Untreated plants when enclosed with treated plants showed leaf abscission. NAA reverses the defoliation effect of ethephon (Morgan, 1969).

It is hoped that the abscission of green leaves of mulberry without injury would reduce the cost of hand labour for harvesting the foliage as silkworm feed (Oyama and Henda, 1969).

Root initiation.—The number of roots on mung bean hypocotyl cuttings was increased but their length was decreased when ethephon was given under continuous illumination (Krishnamoorthy, 1970). Rooting of three cultivars of *Azalea* has also been found to be promoted by ethephon at 1000 ppm (Nell, 1971).

Stimulation of latex flow in rubber.—One of the major problems faced by the rubber industry is the barrier to latex flow, termed 'plugging' which develops soon after tapping. Chemicals such as 2,4–D and 2,4,5–T delay plugging, prolong the period of latex flow and thus enhance the yield. These compounds nevertheless cause some damage to the trees. In the plantation trials (Blencowe, 1971) ethephon applied in palm oil on the bark below the tapping cut was found to have brought about large increases in latex yield and dry rubber content. It is hoped that ethephon will be a more effective yield stimulant and less harmful than 2,4,5–T, although there is no information on the long term effects of ethephon on the tree.

Flowering.—Certain commercially important crops flower rather erratically and interfere with planned production. Induction of flowering and extension of the flowering period help the plant growers in achieving predictable results. One hundred per cent of the pineapple plants can be induced to flower with an application of ethephon at the rate of 1 to 4 lb/acre (Cooke and Randall, 1968). Higher concentrations (2000 ppm) enhance the flowering response and the treated plants mature 4 to 5 weeks earlier than the controls (Randhawa et al., 1970). Foliar application of ethephon at 1000 to 2500 ppm also induces uniform flowering in ornamental bromeliads (Cathey and Taylor, 1970).

Suppression of vegetative growth and promotion of flowering in the ensuing fall or spring seasons have been recorded in ethephon-treated apple and pear trees (Williams, 1970; Clijsters and Porreye, 1970). Recent experiments suggest that, early flowering of mango and pistachio can be achieved by foliar application of ethephon at 1000 ppm (Amchem Technical data sheet, 1970). Ethephon is also reported to control biennial bearing (personal communication from Dr E. K. Chacko).

Treatment with ethephon induces flowering in a qualitative short day plant, *Plumbago indica*, clone 'Angkor', under non-inductive conditions (Nitsch and Nitsch, 1969). *Cannabis sativa* plants given a spray treatment at 240 ppm, come to flower 2-3 weeks earlier than controls (unpublished work of the authors). In the aquatic insectivore, *Utricularia inflexa*, ethephon (10^{-7} M) stimulated the production of a larger number of inflorescences and flowers under inductive conditions in vitro (Mohan Ram et al., 1972). In one experiment even under non-inductive conditions these plants flowered if they were treated with ethephon at 10^{-8} M. Under partially induced conditions ethephon showed a quantitative stimulation of flowering. Flower initiation is delayed by ethephon treatment under inductive condition in *Chrysanthemum* (Rogers and Pang, 1971 and Pang, 1971).

Sex expression. — The mechanism of regulation of sex in plants is one of the challenging problems of flower morphogenesis and differentiation. Hormones have been shown to play an important role in sex expression (Nitsch, 1965). The dioecious nature of *Cannabis sativa* can be altered by ethephon (Mohan Ram and Jaiswal, 1970). Male plants start bearing female flowers when treated with this compound. Various stages of transformation of flowers from male to female sex can be observed. The induced female flowers are functional and produce viable seeds.

Hybrid seed production involves much labour and considerable expenditure. Application of ethephon has shown promise in reducing the cost of emasculation. Single or repeated foliar sprays of ethephon during the first to fifth true leaf stage of cucumber plants resulted in early formation of pistillate or perfect flowers, and decreased or eliminated staminate flowers from the first 15 nodes (Iwahori et al., 1969). The treated monoecious cucumbers resembled the gynoecious cultivars in their pattern of flowering. However, after the fifteenth node, there was a gradual reversion of the plants to the monoecious type (Rudich et al., 1969).

To study the effect of ethephon at various stages of cucumber development, Lower et al., (1970) sprayed ethephon at a concentration of 120 ppm to the monoecious plants having 1, 2, 3, 4, 6, 8, 10, or 12 true leaves. Regardless of the stage of application, the treatment increased the number of pistillate fowers and significantly reduced the staminate flowers.

Treatment with ethephon at three stages of development to andromonoecious, monoecious and hermaphrodite muskmelon cultivars resulted in exceptionally uniform pattern of development, commencing with pistillate flowers, followed by increased frequency of hermaphrodite and male flowers (Karchi, 1970).

George (1971) studied the differential response of six monoecious cultivars of cucumber (*Cucumis sativus*) to ethephon. He found that concentrations between 125 ppm to 500 ppm significantly increased the number of pistillate leaf axils in 4 varieties. The other two varieties showed insignificant response to treatment. He observed that sex conversion in monoecious cultivars of cucumber is affected by the genetic system which controls the female tendency in these cultivars. The species of *Cucurbita* treated with ethephon also showed delay in the appearance of staminate flowers and increase in the number of pistillate flowers (Splittstoesser, 1970).

There are a few varieties of *Vitis vinifera* which produce only staminate flowers and are, therefore, uneconomical. Ethephon treatment results in the induction of functional female flowers which set seeded fruits (Kender and Remaily, 1970).

The present method of hybrid wheat production, using cytoplasmic male sterility has the disadvantage that it will require a system for genetic restoration of fertility in the hybrid. Induction of male sterility by chemicals has been suggested as a means of commercial hybrid seed production. Wheat plants treated with 1000 to 2000 ppm of ethephon at early, mid and late boot stage showed most useable level of functional male sterility without affecting female fertility (Rowell and Miller, 1971).

Thinning of blossoms and immature fruits.—Promotion as well as inhibition of abscission have been utilized in different situations in commercial production of economic crops. Several orchard trees bear enormous quantities of flower, which if allowed to set, would yield an abundance of undersized fruits of poor quality. Blossom or young fruit thinning by chemical means has been a fairly well established practice in recent times. One of the requirements is that any chemical used for flower thinning should have no undesirable after effects like leaf abscission or premature ripening. Successful reports using ethephon have appeared in relation to apple, citrus, pears and grapes (Edgerton, 1968; Weaver and Pool, 1969). In peaches blossom thinning was accomplished by sprays of ethephon at 250-500 ppm without causing severe gumming or dwarfing of subsequent growth (Buchanan and Biggs, 1969; Buchanan et al., 1970).

In the 'Early Amber' (low chilling, short-cycle) peaches, application of ethephon at the stage when the nuclear endosperm was changing to the cellular condition, caused adequate thinning at a concentration of 30 ppm (Buchanan et al., 1970). Depending on the concentration, ethephon application at the prebloom stage caused reduction in fruit set (200 ppm) or total elimination of fruit (2000 ppm) in the apples (Edgerton and Greenhalgh, 1968). Twenty eight and 44 days after full bloom, however, ethephon was ineffective in reducing set. Fruit abscission was promoted when ethephon was applied at maturity. In peaches the fruit size and weight were both increased by ethephon, whereas in apple, reduction in fruit set was also accompanied by reduction in size.

To prevent fruiting in certain trees and shrubs whose fruits may abscise and cause nuisance in one form or the other, repeated applications of high concentrations of ethephon have been found to be successful.

Fruit ripening and maturity. — It is the dramatic ripening effect of ethylene in fruits like the banana and the apple that initiated inquiries into the biological role of ethylene. A vast amount of information has accumulated on the role of ethylene in fruit ripening. Ethephon offers distinct advantages for increasing yields, planning precise harvest timings, concentrating fruit maturity, improving colour and quality, and facilitating mechanical and hand harvesting.

Ethephon at 1000 ppm induced early ripening of green mature bananas to the same extent as ethylene at 100 ppm (Russo et al., 1968). Acceleration of maturity of tomatoes by foliar sprays of ethephon under both glass house and field conditions was reported by several investigators (e.g., Iwahori and Lyons, 1969; Sims, 1969). The size, quality and composition of treated tomatoes resembled those of controls. Ethephon, however, caused a slight inhibition of terminal growth and abscission of flowers and determinancy, if the tomato plants were sprayed at earlier stages. Ethephon at 100 ppm also enhanced the ripening of chilli and pimiento peppers.

In "Early Italian" prunes ethephon enhanced colour development and soluble solids by about 2 weeks when applied 3½ weeks before commercial maturity of untreated fruit (Probsting and Mills, 1969). According to Burg (1962) a fruit must attain a certain minimum stage of maturity before it can be affected by ethylene. However, in the "Early Italian" prunes ethephon was found to be effective over a longer period of time.

In sour cherry cv. Montmorency and certain plums, fruit maturity was advanced and fruit removal force was reduced when ethephon was applied 3–15 days before harvest. However, at concentrations above 2000 ppm, leaf abscission, gummosis, and enlarged lenticels were observed (Bukovac et al., 1969).

Foliar sprays of ethephon (700 to 1400 ppm) on coffee trees with mature green berries, 7 to 11 weeks before the first picking, hastened the main picking periods by 2-4 weeks (Browning and Cannell, 1970).

In plants like fig, peach and grape, fruit growth follows a double sigmoid growth curve. Hastening of ripening by ethephon occurs only if the treatment is given during the transition from period II to III. The treated fruits attain the colour, size and quality equal to those of later maturing controls. In grapes both ethylene and ethephon (dipping of clusters) hasten the onset of ripening but only if applied at restricted periods immediately before the normal time of start of ripening. In cv. Doradillo which has a prolonged slow growth stage, the onset of ripening was advanced by 6 days. But with cv. Shiraz, which has a shorter slow growth period, the advance achieved was only 4 days. When ethephon was applied to Shiraz earlier, it caused a delay in ripening for 3 weeks (Hale et al., 1970).

In contrast with the cost and inconvenience of using gaseous ethylene for post harvest ripening of fruits in specially built chambers, post-harvest dipping of fruits from 30 seconds to 3 minutes in ethephon offers a means of ripening in a wide variety of fruits like bananas, melons, tomatoes, persimmons, pecans, and mangoes. Post-harvest treatment with ethephon has shown promising results with commercial degreening of citrus fruits. In lemons, tangerines, grape fruits and oranges, the rind remains green although the fruits are ripe. Dipping the green fruits in 1000 ppm solution of ethephon for 3 seconds to 10 minutes is sufficient to make them market-ready.

Promotion of fruit abscission. — Certain fruits offer difficulties for mechanical harvesting because they do not reach maturity at the same time and also because of their firm attachment to stem. Ethephon has been shown to reduce the fruit removal force, so that fruits can be harvested by machines with less shaking and injury. Edgerton and Hatch (1969) showed that ethephon increases abscission of mature fruit in some sweet and tart cherries and apples (varieties Rome beauty, Twenty Ounce, Ben Davis). Encouraging results have also been obtained in pears, plums, black currants, gooseberries, black-berries and certain grape varieties by the use of ethephon for machine harvesting (Amchem technical data sheet, 1970).

Application of ethephon to cantaloupe vines 3-5 days prior to hand harvest of the fruit resulted in concentrating fruit maturity and enhancing the number of marketable fruits which could be harvested in 3 successive days (Tyler et al., 1970).

Tissue culture. — Recently ethephon has been incorporated into the plant tissue culture media to find out its effect on growth, cell division and differentiation of tissues. Callus tissues of *Hedera canariensis* showed a substantial increase in growth rate by ethephon treatment. It also promoted tissue cultures of Virginia gold tobacco pith (Stoutemyer and Britt, 1970). Wild carrot tissues cultured with ethephon at a concentration of 10^{-4} M showed inhibition of organization of the tissue but favoured the growth of the callus. This compound disrupts the initiation and maintenance of polarity and hence no organogenesis occurs (Wochok and Wetherell, 1971). The presence of ethephon prolonged the lag phase of cells of *Acer pseudoplatanus* but did not affect the density achieved 28 days after incubation (Mackenzie and Street, 1970).

Other effects. — Fruits of several citrus varieties sprayed with ethephon developed more carotenoid 6 or 7 days after treatment than unsprayed fruit in the flavedo of their rinds. The highest carotenoid increment occurred with rates between 100 and 500 ppm (Young and John, 1971).

The effect of ethephon on photosynthesis and respiration of leaves of young apple trees at various stages of growth has been investigated (Dozier and Barden, 1971). It was found that ethephon treatment upto 4000 ppm failed to show any change in net photosynthesis, whereas it increased the rate of respiration.

It is well established that barley grains show enhanced production of α-amylase in the presence of gibberellic acid. If ethephon is substituted for GA_3, no amylolytic activity can be detected. Ethephon added simultaneously with GA_3 also inhibits the synthesis of α-amylase. Ethephon also inhibits the amylase–starch reaction directly (Devlin and Cunningham, 1970). However, soybean plants treated with ethephon showed increased activity of enzymes like peroxidase and phenylammonialyase (Blomquist, 1971).

Experiments using ^{14}C labelled ethephon have been carried out on many plants to study its translocation, metabolism and ethylene evolution behaviour.

1, 2-^{14}C-(2-chloroethyl) phosphonic acid treatment of the fruits of tomato, cucumber and seedlings of summer squash showed that during the first day, over 21% of the applied ^{14}C-ethephon was converted to ^{14}C-ethylene by the squash plants and 10 to 15% was converted by the tomato plants. One week after treatment, the rate of ^{14}C-ethylene production decreased rapidly to less then 1% per day. After seven days following treatment of tomato leaves, about 15% of ^{14}C was translocated from the site of application to other tissues (Yamaguchi, et al., 1971).

To determine whether or not ethephon accumulates in the edible parts of the fruits, ^{14}C ethephon was sprayed on the foliage of walnut trees. Chromatographic analysis showed that there were no radioactive metabolites of ethephon in the edible kernels (Martin et al., 1971).

Probable Mode of Action

The mode of action of ethephon is related to its ability to release ethylene in plant tissues. The most intriguing aspect of ethylene physiology on which there is little information but much speculation is: how can so simple a molecule affect such diverse activities in a growing plant? We have no clear cut answers, as yet to this question. Results of of some experiments seem to suggest, however, that ethylene might bind with other molecules, perhaps proteins, thereby modifying their conformation and activity, or slot into membranes, altering their permeability or compartmentalizing different cellular organelles.

Future

It is hoped that further work using ethephon as a convenient source of ethylene, would be of much promise in understanding the intricacies of the various aspects of plant growth, development and metabolism in which ethylene is involved. If the long term tests of the application of ethephon should turn out to be without ill effects, it should become one of the most sought–after plant growth regulators in future.

References

Amchem Products Inc. 1967. Amchem 66-329, a new plant growth regulator. *Information Sheet* 37.

——. 1970. Practical applications of ethrel in agricultural prouction. *Information Sheet* 53.

Benedict, W. G., and D. D. S. Thomas. 1969. Effect of 2-chloroethylphosphonic acid and gibberellic acid on survival of tall and dwarf peas grown in root-rot soils. *Can. J. Bot.* **47:** 1659-1662.

Blencowe, J. W. 1971. Recent advances in the stimulation of rubber. *World Crops* **23:** 127-132.

Blomquist, R. V. 1971. *Growth Substance Effect on Soybean Morphology and Physiology*. Ph. D. Thesis. The University of Wisconsin.

Browning, G., and G. R. Cannell. 1970. Use of 2-chloroethanephosphonic acid to promote the abscission and ripening of fruit of *Coffea arabica* L. *J. Hort. Sci.* **45:** 223-232.

Buchanan, D. W., and R. H. Biggs. 1969. Peach fruit abscission and pollen germination as influenced by ethylene and 2-chloroethanephosphonic acid. *J. Am. Soc. Hort. Sci.* **94:** 327-329.

——., R. H. Biggs, J. A. Blake, and W. B. Sherman. 1970. Peach thining with 3-CPA and ethrel during cytokinesis *J. Am. Soc. Hort. Sci.* **95:** 781-784.

Bukovac, M. J., F. Zucconi, R. F. Larsen, and C. D. Kesner. 1969. Chemical promotion of fruit abscission in cherries and plums with special reference to 2-chloroethylphosphonic acid. *J. Am. Soc. Hort. Sci.* **94:** 226-250.

Burg, S. P. 1962. The physiology of ethylene formation. *A. Rev. Pl. Physiol.* **13:** 265-302.

Cathey, H. M., and R. L. Taylor. 1970. Flowering of bromeliads with spray applications of 2-chloroethanephosphonic acid (Ethrel). *Florists Rev.* **146:** 38-39.

Clijsters, H., and W. Porreye. 1970. L' ethrel (CEPA) en culture foruitere. *Fruit Belge* **354:** 16-.

Cooke, A. R., and D. I. Randall. 1968. 2-haloethanephosphonic acids as ethylene releasing agents for the induction of flowering in pineapples. *Nature, Lond.* **218:** 974-975.

Coston, D. C. 1971. Seed germination and seedling growth as affected by pre-harvest applications of ethephon (2-chloroethylphosphonic acid) on red and golden delicious apples. *Hort Science (Abstract)* **6:** 275.

Crocker, W., A. E. Hitchcock, and P. W. Zimmerman. 1935. Similarities in the effect of ethylene and the plant auxins. *Contr. Boyce Thompson Inst.* **7:** 231-248.

Devlin, R. M. and R. P. Cunningham. 1970. Effect of 2-chloroethylphosphonic acid on enzyme induction in barley endosperm. *Econ. Bot.* **24:** 369-373.

Dozier, W. A. Jr., and J. A. Barden. 1971. Net photosynthesis and respiration of apple leaves influenced by (2-chloroethyl) phosphonic acid. *J. Am. Soc. Hort. Sci.* **96:** 798-790.

Edgerton, L. J. 1968. New materials to loosen fruit for mechanical harvesting. *Proc. N.Y. State Hort. Soc.* **115:** 163-166.

——, and W. J. Greenhalgh. 1968 Regulation of growth, flowering and fruit abscission of apples and peaches with Amchem 66-329. *J. Am. Soc. Hort. Sci.* **94:** 11-13.

——, and A. H. Hatch. 1969. Promoting abscission of cherries and apples for mechanical harvesting. *Proc. N. Y. State Hort. Soc.* **114:** 109-113.

Egley, G. H., and J. E. Dale. 1970. Ethylene, 2-choroethylphosphonic acid and witchweed germination. *Weed Sci.* **18:** 586-589.

George, W. L. Jr. 1971. Influence of genetic background on sex conversion by 2-chloroethylphosphonic acid in monoecious cucumber. *J. Am. Soc. Hort. Sci.* **96:** 152-154.

Hale, C. R., B. G. Coombe, and J. S. Hawker. 1970. Effects of ethylene and 2-chloroethylphosphonic acid on the ripening of grapes. *Pl. Physiol.* **45:** 620-623.

Halevy, A. H., R. Shilo, and S. Simchon. 1970. Effect of 2-chloroethanephosphonic acid (Ethrel) on health, dormancy, and flower and corm yield of gladioli. *J. Hort. Sci.* **45:** 427-434.

Iwahori, S., and J. M. Lyons. 1969. Accelarating tomato fruit maturity with Ethrel. *Calif Agri.* **23:** 17-18.

——, J. M. Lyons, and W. L. Sims. 1969. Induced femaleness in cucumber by 2-chloroethanephosphonic acid. *Nature, Lond.* **222:** 271-272.

Iyer, C. P., E. K. Chacko, and M. D. Subramaniam. 1970. Ethrel for breaking dormancy of strawberry seeds. *Curr. Sci.* **39:** 271-272.

Kabachnik, M. I., and P. A. Rossiiskaya. 1946. Organophosphorus compounds. I. Reaction of ethylene oxide wirh phosphorus trichloride. *Izvest. Akad. Nauk S.S.S.R. Otdel. Khim. Nauk* **406:** 295-304.

Karch, Z. 1969. Effect of ethrel (2-chloroethanephosphonic acid) as compared to that of CCC on height and grain yield of spring wheat. *Israel J. Agric. Res.* **19:** 199-200.

——. 1970. Effects of 2-chloroethanephosphonic acid on flower types and flowering sequences in muskmelon. *J. Am. Soc. Hort. Sci.* **95:** 515-518.

Kender, W. J., and G. Remaily. 1970 Regulation of sex expression and seed development in grapes with 2-chloroethylphosphonic acid. *Hort. Sci.* **5:** 491-492.

Ketring, D. L., and P. W. Morgan. 1970 Physiology of oil seeds. I. Regulation of dormancy in Virginia type peanut seeds. *Pl. Physiol.* **45:** 268-273.

Krishnamoorthy, H. N. 1970. Promotion of rooting in mungbean hypocotyl cuttings with ethrel, an ethylene releasing compound. *Pl. Cell Physiol.* **11:** 979-982.

Larsen, F. E. 1970. Promotion of leaf abscission of deciduous nursery stock with 2-chloroethylphosphonic acid. *J. Am. Soc. Hort. Sci.* **95:** 662-663.

Lower, R. L., C. H. Miller, F. H. Baker, and C.L. McCombs. 1970. Effects of 2-chloroethylphosphonic acid treatment at various stages of cucumber development. *Hort. Sci.* **5:** 433-434.

Mackenzie, I. A., and H. E. Street. 1970. Studies on the growth in culture of plant cells. VIII. The production of ethylene by suspension cultures of *Acer pseudoplatanus* L. *J. Expt. Bot.* **21:** 824-834.

Martin, G. C., H. A. Abdel-Gawad, and R. J. Weaver. 1971. Metabolic fate of 1, 2—14. C-(2-chloroethyl) phosphonic acid in walnut. *Hort Sci.* (Abstract) **6:** 309.

Maynard, J. A., and J. M. Swan. 1963. Organophosphorus compounds. I. 2-chloroalkylphosphonic acid as phosphorylating agents *Aust. J. Chem.* **16:** 596-608.

Mohan Ram, H. Y., and V. S. Jaiswal. 1970. Induction of female flowers on male plants of *Cannabis sativa* L. by 2-chloroethanephosphonic acid. *Experientia* **26:** 214-216

——, J. P. Nitsch, and H. Harada. 1972. Studies on growth and flowering in axenic cultures of insectivorous plants III. Effects of photoperiod, ethrel, morphactin and a few other growth substances and metabolic imhibitors on *Utricularia inflexa*. *Z. Pflanzenphysiol.* (In press)

Morgan, P. W. 1969 Stimulation of ethylene evolution and abscission in cotton by 2-chloroethanephosphonic acid. *Pl. Physiol.* **44:** 337-341.

Neljubow, D. 1901. Uber die Nutation der stengel von *Pisum sativum* und einiger anderen Pflanzen *Beih. Botan. Centralbl.* **10:** 128-138.

Nell, T. A. 1971. The effect of several growth regulators on the rooting of three *Azalea* cultivars. *Hort Sci.* (Abstract) **6:** 275-276.

Nitsch, J. P. 1965. Physiology of flower and fruit development. In *Encyclopedia of Plant Physiology* **15:** 1537-1647.

Nitsch, C., and J. P. Nitsch. 1969. Floral induction in short day plant, *Plumbago indica* L., by 2-chloroethanephosphonic acid. *Pl. Physiol.* **44:** 1747-1748.

Oyama, K., and S. Henda. 1969. Abscission acceleration of mulberry leaves by ethylene releasing agent. *Paper presented at the Japan Association. Advancement of Phytoregulators, Shiobara, Japan* Nov. 24-25 (translation).

Pratt, H. K., and J. P. Goeschl. 1969. Physiological roles of ethylene in plants. *Ann. Rev. Pl. Physiol.* **20:** 541-584.

Proebsting, E. L. Jr., and H. H. Mills. 1969. Effects of 2-chloroethanephosphonic acid and its interaction with gibberelic acid on quality of 'Early Italian Prunes. J. *Am. Soc. Hort. Sci.* **94:** 443-446.

Randhawa, G. S., H. C. Dass, and E .K. Chacko. 1970. Effect of ethrel, NAA and NAD on the induction of flowering in pineapple (*Ananas comosus* L.) *Curr. Sci.* **39:** 530-531.

Rogers, M. N., and H. S. Pang. 1971. Effects of ethrel on vegetative growth and floral initiation in *Chrysanthemum morifolium*. *Hort. Sci.* (Abstract) **6:** 292.

Rowell, P. L., and D. G. Miller. 1971. Induction of male sterility in wheat with 2-chloroethanephophonic acid (Ethrel). *Crop Sci.* **11:** 629-631.

Rudich, J., A. H. Halevy, and N. Kedar. 1969. Increase in femaleness of three cucurbits by treatment with ethrel an ethylene releasing compound. *Planta* (*Berl*). **86:** 69-76.

Russo, J. L., H. C. Dostal, and A. C. Leopold. 1968. Chemical regulation of fruit ripening. *Bioscience* **18:** 109.

Sankhla, N., and S. N. Shukla. 1970. Observations on hypocotyl coiling: Effect of ethrel and gibberellin on seedling growth of *Phaseolus radiatus. Z. Pflanzenphysiol.* **63:** 284-287.

Sims, W. L. 1969. Effects of ethrel on fruit ripening of tomatoes : greenhouse, field and postharvest trials. *Calif. Agri.* **23** : 12-14.

Splittstoesser, W. E. 1970. Effects of 2-chloroethylphosphonic acid and gibberellic acid on sex expression and growth of pumpkins. *Physiologia Pl.* **23:** 762-768.

Stoutemyer, V. T., and O. K. Britt. 1970. Ethrel and plant tissue cultures. *Bioscience* **20:** 914.

Thomas, D. D. S., and W. G. Benedict. 1969. Differential effect of 2-chloroethylphosphonic acid on light and dark grown tall and dwarf peas. *Can.* J. *Bot.* **47:** 1725-1726.

Tyler, K., D. May, and R. Miller. 1970 Ethrel sprays reduce number of pickings in hand-harvested cantaloupes. *Calif. Agri.* **24:** 6-7.

Warner, H. L., and A. C. Leopold. 1969. Ethylene evolution from 2-chloroethylphosphonic acid. *Pl.Physiol.* **44:** 156-158.

Weaver, R. J., and R. M. Pool. 1969. Effect of ethrel, abscisic acid and a morphactin on flower and berry abscission and shoot growth in *Vitis vinifera. J. Am. Soc. Hort. Sci.* **94:** 474-478.

Williams, M. W. 1970. Post harvest application of growth regulators to increase spur formation and subsequent flowering and fruiting of young apple trees. *Hort Sci.* (Abstract) **5:** 349.

Wochok, Z. S., and D. F. Wetherell. 1971. Suppression of organized growth in cultured wild carrot tissue by 2-chloroethylphosphonic acid. *Pl. Cell Physiol.* **12:** 771-774.

Yamaguchi, M., C. Wanchu, and S. F. Yang. 1971. The fate of ^{14}C (2-chloroethyl) phosphonic acid in summer squash, cucumber and tomato. *J. Am. Soc. Hort. Sci.* **96:** 606-609.

Young, R., and O. John. 1970. Ethylene-induced carotenoid synthesis in *Citrus fruit* rinds. *Hort Sci.* (Abstact) **6:** 303.

Zimmerman, P. W., and F. Wilcoxon. 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. *Cont. Boyce Thompson Inst.* **7:** 209-229.

Adv. Pl. Morph. 1972 : 315–324

ROLE OF INSOLUBLE POLYSACCHARIDES IN THE ANTHER DEVELOPMENT OF SOME MEMBERS OF GRAMINEAE-MILLETS

By M. G. Panchaksharappa and C. K. Rudramuniyappa

Department of Botany, Karnatak University, Dharwar-3, India

ABSTRACT

Tissue constitution and its differentiation is basically biochemical in plant organization and the role of insoluble polysaccharides in it is equally conspicuous as assessed in the anther sections of some four members of Gramineae (Millets) employing Periodic acid Shciff's method (Jensen, 1962). In the tissue organization insoluble polysaccharides appear in the form of cell wall and cytoplasmic PAS positive tinge of variable concentrations specific to the tissue as well as growth phase and the plant, and also as storage starch.

Based on these facts, it is possible to descern two developmental categories of tissues in the tissue organization of anther. They are embryonic tissues which are radically different from the permanent tissues in their polysaccharides constitution, although the former precedes the latter in growth and differentiation. The embryonic tissue is characterized by very thin PAS positive cell wall and varying concentrations of cytoplasmic PAS positive tinge although macromolecular in nature. The embryonic tissue transcends into permanent tissue by way of biochemical differentiation developmentally, as observed in all the tissues of anther derived from the archesporium. But tapetum stands as an exception to this morphogenetic phenomenon, as it remains thin walled resembling the sporogenous tissue and the spores in its cytoplasmic PAS positive tinge until it degenerates, thin walled. The permanent tissue is characterised by thick PAS positive cell walls and/or storage starch as seen in epidermis, endothecium, middle layer, pollen and the connective.

Pollen mother cells and the spores are exception to this. They develop thick transitory additional spore mother wall which extends around the dyads and tetrads also. However, it disintegrates after the completion of meiosis. Therefore, this additional wall cannot be equated with the cell wall of the permanent tissue, and it appears to play a vital role in meiosis acting as a "molecular filter", aided by tapetum.

The connective also differes from the permanent tissue markedly. It contributes to the inner wall layer and in turn to the tapetum, by regaining thin walled nature and losing starch storage in the area. This change over is an act of dedifferentiation, a morphogenetic phenomenon.

Rise in the cytoplasmic polysaccharides in the microspore is followed by starch storage in pollen. The thin intine is PAS positive and appears thick at germ-pores. PAS positive endothecial thickenings appear with the dissppearance of starch in it and this differentiation coincides with the pollen formation.

INTRODUCTION

The reproductive structures, as understood embryologically, are complex in their organization, specially anther and ovule. The complexity is obviously due to varied tissues that are differentiated during the ontogeny. But vast embryoological facts available have not been able to reveal the basic causal factors responsible for tissue organization in them. It is known beyond doubt that the ontogenetic differentiation of tissues is basically biochemical. Therefore, the biochemical assessment of tissues in the reproductive structures would obviously reveal some of the causal factors underlying such a complex organization.

For this purpose, extraction procedures have been tried on the fresh materials of anther and ovule in order to assess total quantity of the substances present in the extract. But those methods have not helped in accounting for the histochemical constitution and the differentiation of individual tissue contained in the anther, ovule or seed. Therefore, in this regard, histochemical assessment of tissue-sections appears to be the only way out. Of late, attempts have been made on the tissue sections employing autoradiography and optical devices such as: electron, florescent and phase-contrast as well as light microscopes.

Botanists do not seem to be that enthusiastic in assessing the histochemical constitution of the tissue sections of the fresh materials, as it involves many technical complications and the progress achieved is slow and time consuming when compared to the fixed material studies. Known paraffin method of tissue sectioning is employed in the latter. But the latter method enables the assessment of only the macromolecular insoluble substances namely, polysaccharides, nucleic acids, proteins and lipids which are mostly metabolites. The present account pertains to the assessment of insoluble macromolecular polysaccharides in the tissue sections of anther at some developmental growth phases. The members selected for the work belong to the Gramineae (Millets) which are common food crops in India.

A few stray reports are available on the histochemistry of reproductive structures in tissue sections pertaining to the role of polysaccharides. Among these, notable contributions are by Heslop Harrison (1963, 1964) on *Salina pendula* and *Cannabis sativa* and De Fossard (1969) on *Chenopodium rubrun*. They have observed special spore mother wall (Beer, 1911 in Heslop-Harrison, 1963) which has been identified as callose, but negative to Periodic acid Schiffs reaction. This wall is presumed to aid in meiosis acting as a molecular filter. Recent reports of Heslop-Harrison are on the chemical nature of the pollen wall (1968) and the occurrence of storage hydrolytic enzymes in the cellulose intine, specially at the sites of germ pores (Knox and Heslop-Harrison, 1969). De Fossard (1969) has analysed the chemical nature of endothecial thickenings employing the extraction procedures and the probable cause for the delayed differentiation of the thickenings, which being the inhibitory products of tapetum, during the early period of anther growth. Other contributions are on the storage of starch in the anther wall layers and the pollen (Milyaeva and Tsinger, 1968), in tapetum of wheat (Joppa et al., 1960 in Goss, 1968) and on the occurrence of PAS positive cell wall around the male cells (Cass and Jensen, 1970).

MATERIALS AND METHODS

The food crops selected for the present work were *Panicum miliaceum* L. (Proso millet), *Pennisetum typhoideum* Rich. (Pearl millet), *Setaria italica Beau.* (Foxtail millet) and *Triticum durum* Desf. (Wheat). The seeds of the first three plants were obtained from I. A. R. I., Delhi and those of *Triticum durum* Desf. from the local source. They were sown in the university experimental garden. The flowering buds and the flowers were fixed in FAA and Cornoy's fluids for 24 hours and 3 hours, respectively. The routine embryological procedure were used employing n-butanol series for dehydration and paraffin secseries for dehydration and paraffin sectioning. The sections were cut at 8 μ and mounted in gelatin adhesive.

The detailed procedures used for the preparation of stains and for staining the tissues were mainly from the "Botanical Histochemistry" (Jensen, 1962). To localise insoluble polysaccharides periodic acid Schiff's method-commonly known as PAS method-was adopted. For oxidation, 0.5% aqueous periodic acid solution was used on the tissue sections at room temperature for 10 to 20 minutes. The sections were rinsed in water and stained with Schiff's reagent for about 10 to 20 minutes. The staining was allowed by rinsing of the slides in water and subjecting them to the bleaching for one or two minutes. The bleaching agent was prepared by using 5 ml of HCl and 5 ml of 10% sodium or Potassium metabisulphite in 90 ml of water. Finally, the slides were dehydrated using alcohol and xylol series and mounted in canada balsam.

Results.—The cell walls and the storage starch invariably take purple-red megenta colour. The polysaccharides when present in the cytoplasm and nuclei also show purple colour indicating macromolecular insoluble substances.

Control tests.—For the conformation of PAS method, the following control tests were conducted:

(a) Avoidance of periodic acid in the said procedure (Cass and Jensen, 1970).

(b) Acetylation method for prevention of PAS reaction (Lillie, 1954 in Pearse, 1960).

(c) IKI test for starch.

Extraction procedure.—The method was adopted to extract callose from the cell wall as it dissolves in the solutions of calcium chloride or stannous chloride (Johansen, 1940) or potassium hydroxide (Northcote, 1969).

The present assessment of the histochemical constitution of the anther tissues, with particular reference to their polysaccharides content in tissue sections, is mainly qualitative. However, semiquantitative assessment was also made based on the comparative consideration of the relative concentrations, as observed under the light microscope. For convenience in describing the results, concentrations of cyctolasmic polysaccharides are graded into faint, normal, rich and intense based on stainability. The concentrations adjudged at less than faint is described as negligible. The experimental results are presented according to the developmental phases of growth, and the similar stages of the plants assessed are compared.

OBSERVATIONS

The anther primordium which is encovered by the epidermis of floral apex is a protuberance of homogeneous tissue in which archesporium differentiates (Figs. 1, 2). During the period of differentiation of the archesporium, the primordium appears thin walled having less tinge of cytoplasmic polysaccharides, while its subtending area exceptionally shows the storage of starch (Fig. 1). *Panicum miliaceum* (Fig. 1) differs from

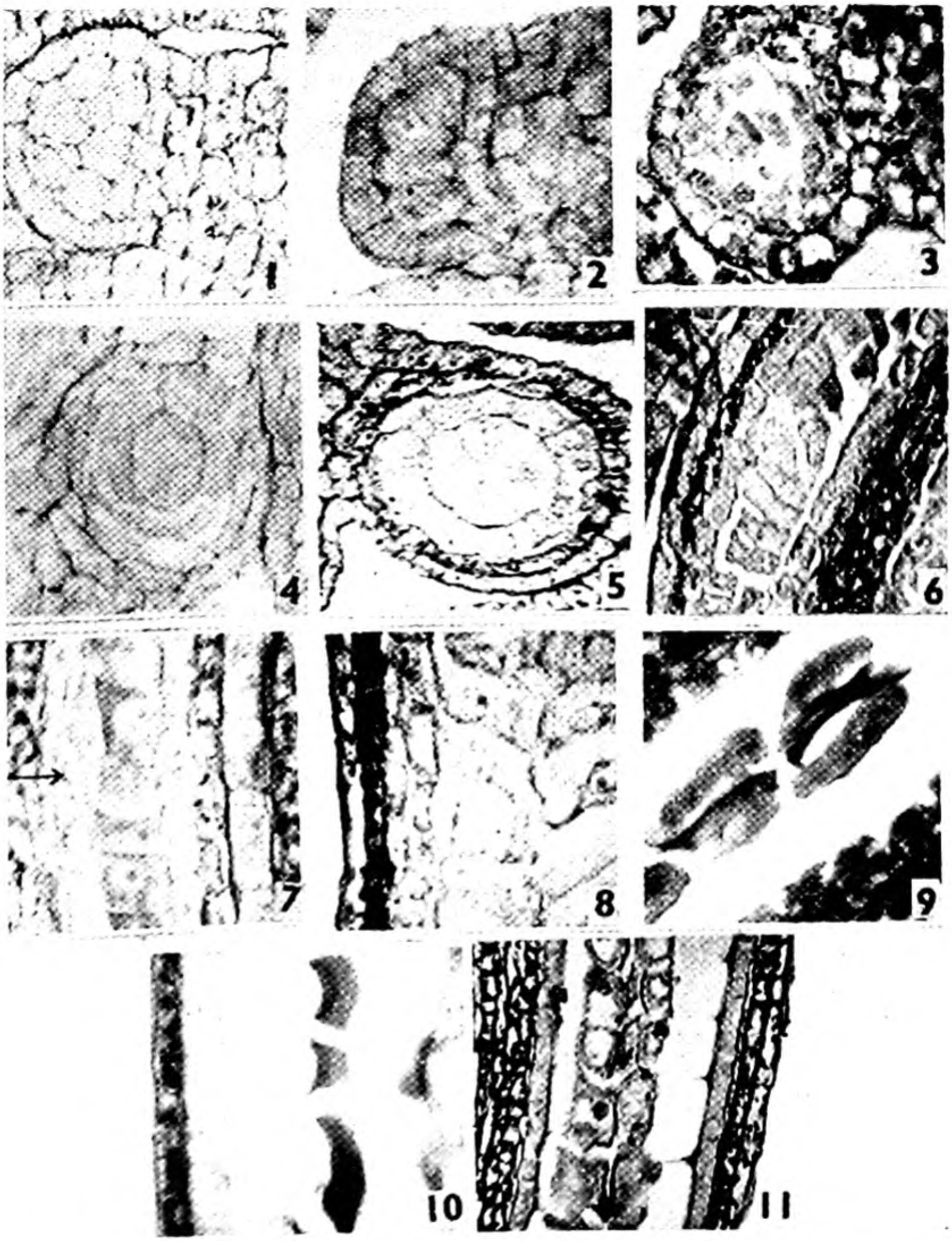
Setaria italica (Fig. 2) in having normal PAS positive tinge in the cytoplasm indicating the specific difference between the species. It is known that the archesporium which is the primary source for the differentiation of all the tissues of anther wall, including tapetum and the sporogenous tissue, the differentiation is biochemical and happens during the ontogeny of anther of successive growth phases. The connective contributes its share to meet this end being a storage tissue for starch which is a source of energy for the anther development.

In a young anther at an early sporogenous tissue wherein endothecium, inner wall layer and the sporogenous tissue are formed, variation in the concentration of cytoplasmic polysaccharides was noticed among the plants examined, although all tissues in a plant showed uniformity in the stainability (Figs. 3, 4). *Panicum miliaceum* (Fig. 3) showed a marked rise in the PAS positive tinge of the cytoplasm, even resulting in the storage of starch in endothecium, but richer in the connective. *Setaria italica* (Fig. 4), on the contrary, maintained a negligible cytoplasmic tinge even in the cell walls of these tissues indicating that growth and differentiation of anther tissues could be effected with lesser metabolic activity.

At late sporogenous tissue, anther tissues show further change in their histochemical constitution. The endothecium which has grown shows greater increase in starch storage and the connective also has enriched itself, parallely in this regard (Figs. 5-7). Both inner wall layer and the sporogenous tissue show uniformity in their histochemical constitution, although they increase in their cell size and the tissue area. The concentration of cytoplasmic polysaccharides in them is faint as observed in *Panicum miliaceum* (Fig. 7) and *Pennisetum typhoideum* (Fig. 5) but at normal level in *Triticum durum* (Fig. 6). At this, connective tissue appears to show marked deviation in its polysaccharide content and in the behaviour of its cell which are neighbouring the sporogenous tissue, as observed in *Panicum miliaceum* (Fig. 7) and *Triticum durum* (Fig. 6). These cells become devoid of their starch storage and attain thin walled nature. PAS positive tinge in the cytoplasm is also reduced to a faint level. Such a transformation in the biochemical constitution of these cells appears to be a secondary happening by way of dedifferentiation. These cells of the connective become one with the inner wall layer of the anther which is a vital tissue as it contributes to tapetum by a periclinal division of its cells.

Differentiation of tapetum from the inner wall layer marks the begining of successive growth phases of the anther when the pollen mother cells (PMCs) are coined (Figs. 8-11). Both the derivative tissues of the inner wall layer namely, middle layer and the tapetum appear alike when young in their cell size and cytoplasmic polysaccharide content (Fig. 8) During the subsequent growth phases, middle layer shows no active growth, while the tapetum enlarges greatly. Subsequently, the former appears insignificant and rarely shows the presence of starch grains. Parallely with the tapetum, spore mother cells also enlarge leading to maturity-preparatory to meiosis, while the endothecium is further enriched with starch storage and even the epidermis shows starch deposition in continuity with the connective tissue (Fig. 8-11).

In the spore mother cells, as they grow leading to maturity, additional spore mother cell wall is formed around the pollen mother cells (Figs. 9-11). This



FIGS. 1-11. Sections of anther tested with Periodic acid Schiff's (PAS) method for polysaccharides. Figs. 1, 2. *Panicum milliaceum* and *Setaria italica*. Anther primordia showing the difference in their PAS positive tinge, normal in Fig. 2. Fig. 1, x 900; Fig. 2, x 1000. Figs. 3. 4. *Panicum miliaceum* and *Setaria italica*. At early sporogenous tissue. Note an increase in the cytoplasmic polysaccharides in Fig. 3. Fig. 3, x 500; Fig. 4, x 1500., Figs. 5, 6. *Pennisetum typhoideum* and *Triticum durum*. The diffrerentiation of edothecium is marked with the appearance of starch. Fig. 5, x 500; Fig. 6, x 400. Fig. 7. *Panicum miliaceum*. Older sporogenous tissue. Note the difference in the, stainability of the connective cells arrow, x 700. Fig. 8. *Pennisetum typhoideum*. Tapetum is differentiated, x 1000. Figs. 9-11. *Panicum miliaceum, Pennisetum typhoideum* and *Triticum durum*. At pollen mother cells. Note the contrast in the cytoplasmic polysaccharides and the additional thickening around the PMCs. Fig. 9, x 900; Fig .10, x 600; Fig. 11, x 400.

additional thickening is deeply PAS positive as indicated by its intense tinge which is richer towards the locule. At this, even the cytoplasm of PMCs shows variation in the polysaccharide content resembling tapetum in this regard. The concentration increases to rich level in *Panicum miliaceum* (Fig. 9), remains normal in *Triticum durum* (Fig.11), and is reduced to negligible quantity in *Pennisetum typhoideum* (Figs. 8, 10).

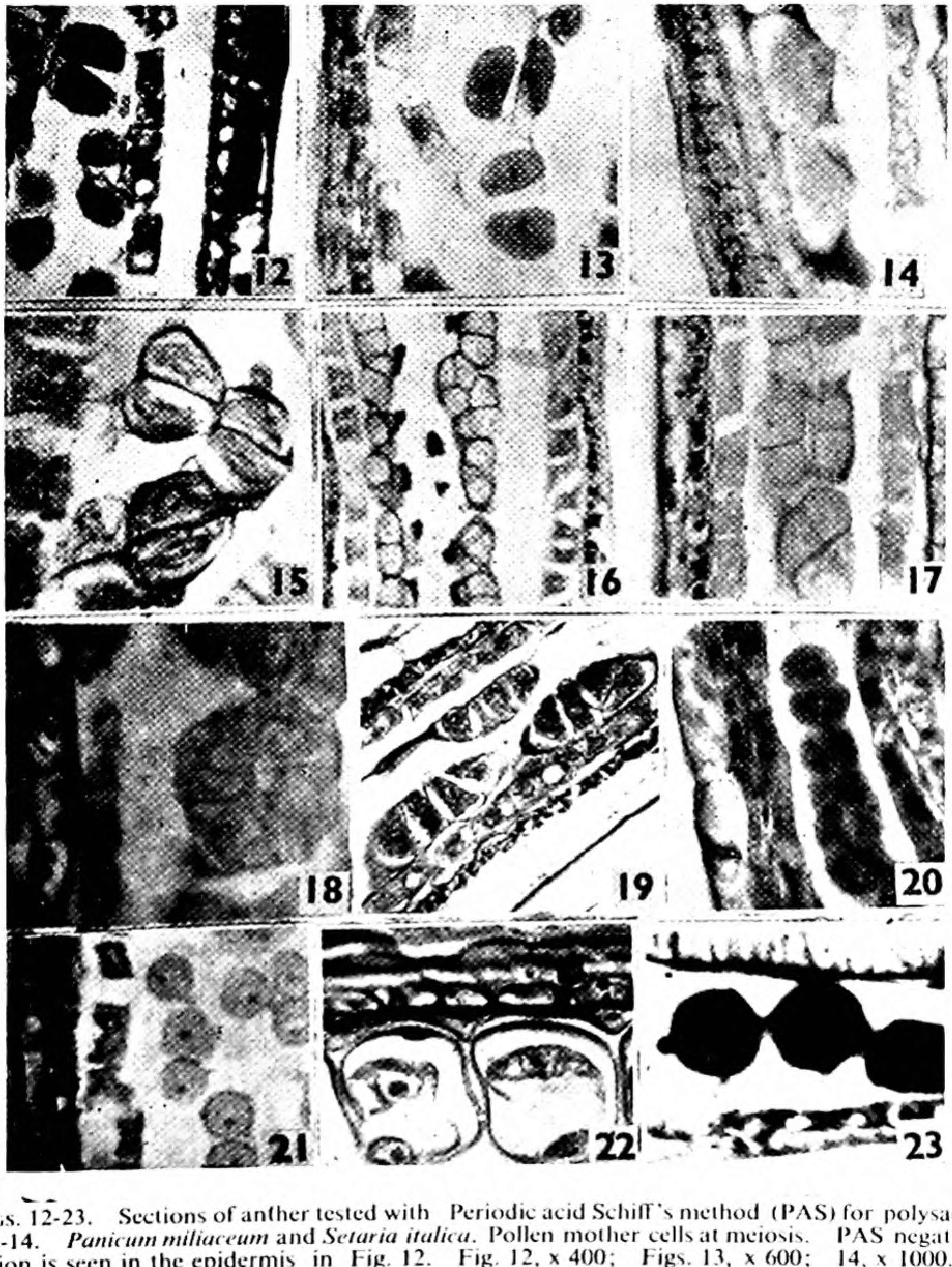
During the period of meiosis, both tapetum and the derivatives of meiocytes namely, dyads and tetrads resemble very much in the concentration of polysaccharides (Figs. 12-19). However, the cytoplasm appears richer during meiosis I than in meiosis II. The spindles and the chromosomes also show PAS positive tinge (Figs. 12, 13). It is interesting to note that the additional wall of spore mother cells is seen extended around the cells of dyads and tetrads and persists with varying degree of thickness among the plants studied (Figs. 12-19). During this period when the microspores are formed, starch content of anther wall layers including [illegible] remains unchanged and no [illegible] the differentiation of endo-[illegible]enings could be observed. [illegible] miliaceum* epidermis [illegible] of brown PAS negative [illegible] persists (Figs. 12, 9).

[illegible] meiosis brings about trans[illegible] the constitution of anther tissues. The spores of a tetrad separate, become round and develop thin PAS positive intine, followed by thick exine (Figs. 20-22). The additional special spore wall has disintegrated by then. The plants show variation in the concentration of cytoplasmic polysaccharides of the microspores: rich in *Panicum miliaceum* (Fig. 20), but faint in *Setaria italica* (Fig. 21). Tapetum resembles the microspores in the polysaccharide content. In the two-celled pollen grains of *Pennisetum typhoideum* (Fig. 22) both vegetative cell and the peripheral lenticular generative cell show normal concentration of polysaccharides. The intine bears PAS positive tinge, thick at the germ pores.

The shedding 3-celled pollen, however, shows rich starch storage in *Panicum miliaceum* (Fig. 23). Same is the case with all the plants examined. At this, endothecial layer has lost its starch storage, so also the epidermis. In place of it thick PAS positive endothecial thickenings are differentiated, but not in the epidermis (Figs. 22, 23). Tapetum no longer persists healthily at this stage, as it degenerates without showing any change in its polysaccharide content. It never becomes thick walled, perhaps because of the fact that it does not constitute itself into a permanent tissue unlike the neighbouring endothecium and the connective tissues. Even the sporogenous tissue and the spores remain thin walled until the pollen are formed.

DISCUSSION

Polysaccharides are the biochemical substances of primary importance in plant organization and occur in the form of soluble sugars, cell wall thickening and storage starch. They constitute body building materials of varied nature and play a central role in the biochemical energetics and in the production of ATP. The presence of starch in tissues is indicative of the stored energy needed for their growth and differentiation. Therefore, unlike histologists, histochemists are interested in the presence of starch and its contribution to the tissue organization. In addition, the insoluble polysaccharides are also seen in the form of cytoplasmic PAS positive tinge of varied concentrations



FIGS. 12-23. Sections of anther tested with Periodic acid Schiff's method (PAS) for polysaccharides. Figs. 12-14. *Panicum miliaceum* and *Setaria italica*. Pollen mother cells at meiosis. PAS negative brown deposition is seen in the epidermis in Fig. 12. Fig. 12, x 400; Figs. 13, x 600; 14, x 1000. Fig. 15. *Pennisteum typhoideum* at dyad showing additional thickening around the dyad cells, x 800. Figs. 16-18. *Pennisetum typhoideum*, *Setaria italica* and *Triticum durum*. At tetrad. Note the additional thickening around the spores and starch in the epidermis in Figs. 17, 18. Fig. 16. x 300; Fig. 17, x 600; Fig. 18, x 800. Figs. 19, 20. *Panicum miliaceum* at successive growth phases of tetrad. Fig. 20 shows a rise in the cytoplasmic PAS positive tinge. Note the absence of additional spore cell wall. Fig. 19, x 750; Fig. 20, x 400. Fig. 21. *Setaria italica* at microspores. Both microspores and tapetum show faint cytoplasmic PAS positive tinge, x 800. Figs, 22, 23,. *Pennisetum typhoideum* and *Panicum miliaceum* at pollen formation. Endothecial thickenings are differentited. Observe the absence of starch in 2-celled pollen in Fig. 22. Fig. 22, x 2200; Fig. 23, x 1500.

in the fixed materials. Therefore, it is reasonable to think that the extranuclear histochemical substances such as insoluble polysaccharides, play an important role in tissue organization, as observed in the present work on millets, when identical DNA-coded chromosomes in the plant tissues do not seem to help in understanding about the causal factors in tissue organisation (Buckley, 1969).

It is observed that the embryonic tissues in anther are characterised by thin PAS positive cell wall and the cytoplasmic tinge of varied concentrations-specific to the plant, to the tissue and the stage of development. Such tissues are liable for histochemical transformation at successive phases of an—ther development as it happens in anther wall layers, tapetum and the sporogenous tissue, until they reach permanancy, and again in endothecium and pollen formation. On the contrary, the permanent tissues develop thick PAS positive cell walls and often store starch. In this context it is interesting to note that both tapetum and the sporogenous tissue show marked [illegible] in being thin walled and having [illegible] concentration of PAS positive [illegible] polysaccharides specific to [illegible] exception to these generali- [illegible] observed in the thick PAS [illegible] spore mother wall and [illegible] the connective that contri- [illegible] tapetum (Fig. 7).

Dep[illegible] of an additional special spore mother cell wall (Beer, 1911) appears to be a universal feature of the spore mother cells, as observed in millets (Figs. 9-11) and also in *Salina pendula* and *Cannabis sativa* (Heslop-Harrison, 1963, 1964) and *Chenopodium rubrum* (De Fossard, 1969). This thickening appears prior to meiosis and persists even during the period of spore formation. Heslop-Harrison (1963, 1964) has identified the thickening as callose which belongs to general polysaccharides, employing Aniline-blue staining method and florescence microscope. But in millets, it is observed to be PAS positive. To confirm this observation, extraction procedure was adopted. As callose dissolves in calcium chloride or stannous chloride (Johansen, 1940) and also in potassium hydroxide (Northcote, 1969), anther sections at the spore mother cell stage were subjected to the treatment with the above said reagent, potassium hydroxide. Even after the extraction of callose, the special spore mother cell wall continued to be stained thick with Periodic acid Schiff's stain. The said control test, therefore, confirms that the additional wall is not of callose alone, if the callose present in it has dissolved in the reagent. However, it is mainly made of polysaccharides being richly PAS positive.

Meiosis is the phenomenal happening in the spore mother cells which results in the differentiation of haploid spores. The special spore mother cell wall exists during the period of meiosis and disintegrates when the spores separate out from a tetrad. Therefore, it is reasonable to think that this additional wall is intimately connected with meiosis. Heslop-Harrison (1963, 1964) has rightly described this wall as a molecular filter meant to allow basic nutrients to pass into the spore mother cells through the passage of this callose wall which is PAS positive wall in millets, in order to effect the formation of haploid generations. His account on this implies that the source of basic nutrients is tapetum. If so, obviously there exists a histochemical correlation between the tapetum and the pollen mother cells in bringing out haploid spores. This possibility seems to gain support, as the tapetum degenerates after the formation of microspores. However, the recent view of Moss and Heslop-Harrison (1967), based on their observations made using microdensitometry, indicates that the relationship between the tapetum and the sporogenous tissue is to be still regarded as obscure.

The connective tissue is a storage reservoir for the anther tissues and therefore, a main source of energy for the anther development. The storage is lost finally when the pollen are formed and the endothecial thickenings are differentiated. Contribution of the connective to the inner wall layer, prior to the defferentiation of the latter into tapetum, is a morphogenetic happening connected with sporogenesis. Due its contribution, the tapetum differentiated from the inner wall layer encovers the sporogenous tissue prior to meiosis, even on the connective side. The inner wall layer, prior to its contribution to tapetal layer, shows faint or negligible PAS positive cytoplasmic polysaccharides and thin cell walls indicating its embryonic nature. Similarly, the connective cells contributory to the inner wall layer differentiate again into very thin-walled cells losing starch storage in them and acquiring faint PAS positive cytoplamic tinge (Fig. 7). This happening is a de-differentiation, a morphogenetic phenomenon and speaks of the necessity of the sporogenous tissue for tapetum on the connective side also. Therefore, it appears that histochemical correlation exists between the tapetum and the spore mother cells perhaps for the basic nutrients which pass through the molecular filter, as also thought by Heslop-Harrison (1964). These possibilities are based on the circumstantial evidences only, although they need further verifications. This problem is being tackled further, here.

It is known that the anther dehiscence takes place assisted by the endothecial thickenings. The nature of these thickenings has not been fully understood. With periodic acid Schiff's reagent, they take rich stain indicating polysaccharides nature. De Fossard (1969) has analysed the nature of these thickenings in *Chenopodium rubrum* by employing extraction procedure and has identified them as mainly of alpha-cellulose nature, unlike the other wall layers which contain mainly pectin, hemicellulose and noncellulosic polysaccharides. Although De Fossard (1969) has found callose as a transient material in these endothecial thickenings, he has not detected lignin in them. In millets, these thickenings give positive reaction with Azur B (Jensen, 1962) indicating lignin content in them (unpublished).

Prior to the differentiation of endothecial thickenings, the endothecium stores rich quantity of starch which is lost, perhaps consumed, with the expression of endothecial thickenings in the layer. Therefore, it is possible that the storage starch in the layer persists as a basic metabolic requirment for the differentiation of endothecial thickenings which occur during the pollen formation. The time of occurance is perhaps morphogenetically controlled. Whether it is controlled by the tapetum (De Fossard, 1969) or not needs strengthening evidence. The neighbouring layer epidermis also stores starch in millets and seems to assist endothecium in its role, but the epidermis itself does not develop any thickenings of the kind.

REFERENCES

BUCKLEY, E. H. 1969. Regulation of male gametogenesis. A Search for new bioregulants. In *Current Topics in Plant Science*. J. E. Gunckel. (ed.). Acad. Press. New Yrok.

CASS, D. D., AND W. A. JENSEN, 1970. Fertilization in barely. *Am. J. Bot.* **57**: 62-70.

De Fossard, R. A. 1969. Development and histochemistry of the endothecium in the anthers of *in vitro* grown *Chenopodium rubrum* L. *Bot. Gaz.* **130:** 10-22.

Goss, J. A. 1968. Development, physiology and biochemistry of corn and wheat pollen. *Bot. Rev.* **34:** 333-358.

Heslop-Harrison, J. 1963. Ultrastructural aspects of differentiation in sporogenous tissue. *Symp. Soc. Expt. Biol.* **17:** 315-340.

——. 1964. Cell walls, cell membranes and protoplasmic connections during meiosis and pollen development. In *Pollen Physiology and Fertilization.* H. F. Linskens. (ed.). North-Holland, Amsteram. 39-47.

——. 1968. Wall development within the microspore tetrad of *Lilium longiflorum. Can. J. Bot.* **46:** 1185-1192.

Jensen, W. A. 1962. *Botanical Histochemistry.* Freeman, San Francisco.

Johansen. D. A. 1940. *Plant Microtechnique* MacGraw-Hill, New York.

Knox, R. B., and J. Heslop-Harrison. 1969. Cytochemical localization of enzymes in the wall of the pollen grain. *Nature, Lond.* **223:** 92-94.

Moss, G. I., and J. Heslop-Harrison. 1967. A cytochemical study of DNA, RNA and Protein in the developing maize anther. II. Observations. *Ann. Bot. N. S.* **31:** 555-572.

Milyaeva, E. L., and N. V. Tsinger. 1968. Starch in developing anthers of *Citrus sinensis:* a cytochemical and electron microscope study. *Fiziologiya Rast.* **15:** 303-307.

Northcote, D. H. 1969. Synthesis and metabolic control of polysaccharides and lignin during the differentiation of plant cellls. *In Essays in Bochemistry* P. N. Cambell and G. D. Greville. (ed.). Vol. 5. Acad. Press.

Pearse, A. G. E. 1960. *Histochemistry. Theoretical and Applied.* Little Brown and Co. Boston.

Adv. Pl. Morph. 1972: 325-338.

MORPHOGENETIC EFFECTS OF GROWTH SUBSTANCES ON *REGNELLIDIUM DIPHYLLUM*

BY A. N. RAO

Botany Department, University of Singapore, Singapore-10

ABSTRACT

Starting from the early embryo stage plants of *R. diphyllum* are grown in aseptic cultures under controlled temperature and light conditions. IAA, NAA, 2, 4-D and GA are used in four different concentrations (100-.1 mg/L). At higher concentrations the plants are deformed showing variations in general morphology. Morphogenesis of proliferations or callus outgrowths produced by rhizome tissues are examined. At lower concentrations the experimental plants resemble the controls but show variation in the number of leaves and roots produced. The morphogenesis and anatomical structure of various organs are studied in comparison with those of control plants. Variations in position and development of axillary buds as influenced by growth substances are considered. Origin and structure of fasciated roots are described. The correlation between normal as well as abnormal structures and the diversion of materials into the growth of these various structures are explained. These findings are discussed in relation to the earlier observations made on the invitro cultures of other aquatic ferns.

INTRODUCTION

In vitro cultures of whole and intact plants have been used in several investigations with various objectives, and the results of such studies are recently summarised (See Allsopp, 1964 for details). Aquatic or amphibious plants, both of angiosperms and ferns, have been particularly useful in such experimental studies. Among the ferns, *Marsilea*, *Regnellidium* and *Salvinia* are very suitable which can be grown from the embryo stage to the mature plants in liquid cultures and therefore, they are used in experimental studies (Allsopp, 1952; Rao, 1966a; Gaudet and Koh, 1968). Among angiosperms *Utricularia*, and other water plants are often used (Pringsheim and Pringsheim, 1962, 1967; Mohan Ram et al, 1971). In the past, several workers have studied different aspects of morphogenesis and growth physiology of *Marsilea*, *Regnellidium* and considerable number of publications have resulted (Allsopp, 1963; Gaudet, 1964, 1964a, 1965, 1965a, 1967; Rao, 1966a; White, 1966, 1968). The morphogenetic effects of sugars and mannitol on *Regnellidium diphyllum* Lind. have been described before and that both the kind and concentrations of different sugars can affect the morphology, size and anatomy of such plants (Rao, 1966a;). In the present paper, the effects of various auxins and gibberellic acid on the growth morphology and anatomy of *R. diphyllum* are described.

MATERIALS AND METHODS

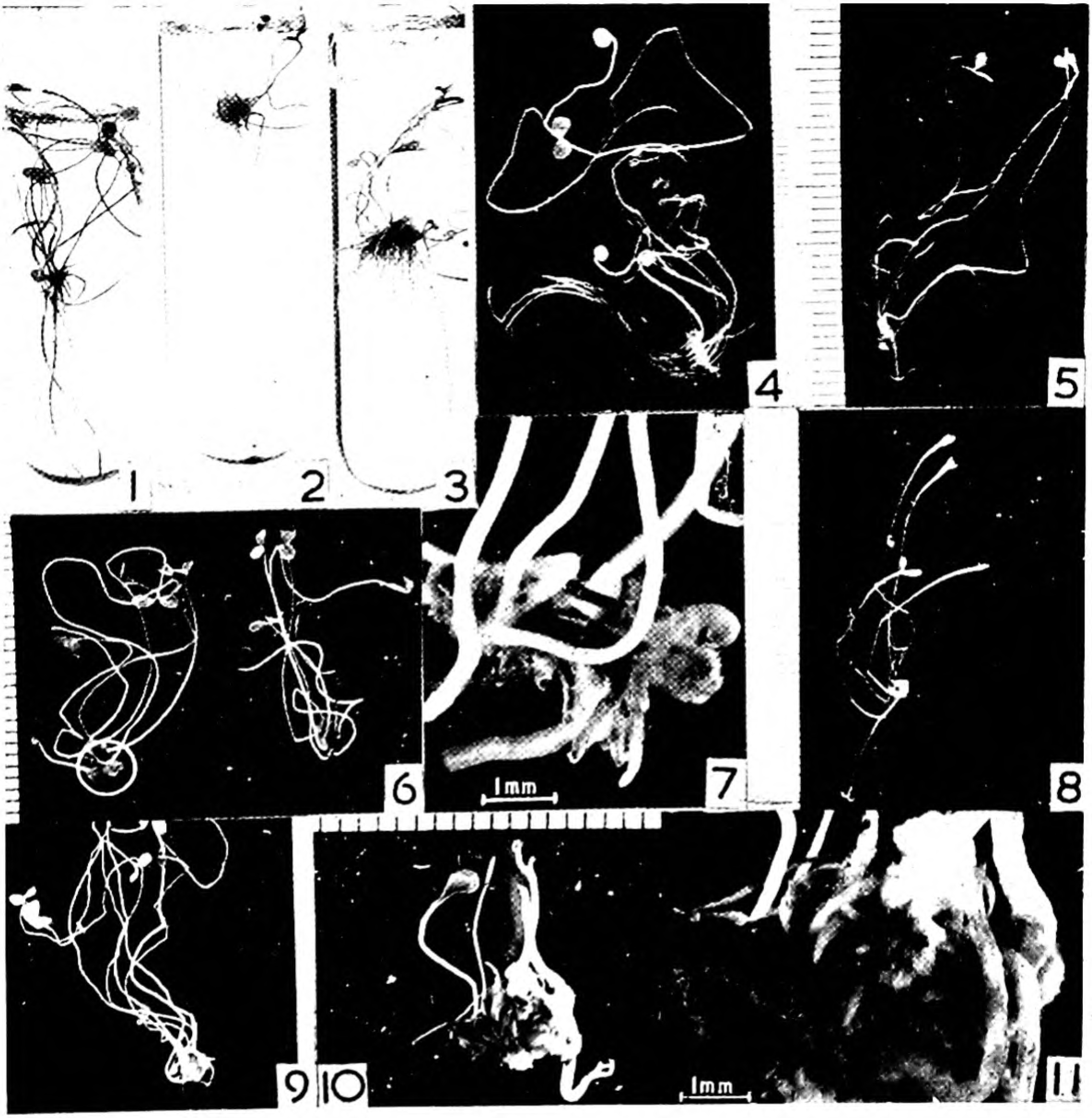
The basic medium consisted of Knop's

solution (half strength) prepared with glass distilled water. To one litre of this medium 10 gm. of glucose (B. D. H. Analar), 1 ml. of trace element solution (Nitsch, 1951) and 1 ml. of 0.1% ferric citrate solution were added. This formed the control medium. The well known physiologically active substances like Indole-3-acetic acid (IAA), —naphthalene acetic acid (NAA), 2,4-dichlorophenoxy acetic acid (2, 4-D) and gibberellic acid (GA) were used individually at different concentrations. .1, 1, 10 and 100 mg/L. pH of the medium was adjusted to 6.0 before autoclaving. Each experiment was repeated at least twice and the observations were based on 10-12 cultures per treatment. All the experiments lasted at least for 12 weeks. Nearly 18 sporocarps were used and about 50% of them were found to be abortive. The micro—and megaspores were released but there was no female gametophytic tissue formation at the apical region of megaspores. In some cases the green tissue was noticed but there was no fertilization. The growth rate was uniform in 95% of the sporelings that developed from megaspores of any one sporocarp. Equal number of cultures were maintained in light and dark conditions. Each sporocarp consists, on an average, 142 megaspores and innumerable number of microspores. All the sporelings used in any one experiment for control as well as to study the effects of either auxins or GA came from a single sporocarp. The procedure followed for the preparation of sterile cultures and conditions for their growth were similar to those described earlier (Rao, 1966a:). In the earlier experiments the young sporelings 3-5 days old were transferred to the auxin media directly along with the controls. Especially at higher concentrations (100 mg/L) the majority of these sporelings ceased to grow further, and obviously this concentration was toxic. In order to avoid this initial mortality, the autoclaved auxin solutions were added to the 8-10 day old cultures. By this time the young sporelings showed two leaves and two roots, and the sporelings continued to grow after treatment and the variations of their growth and morphological characters are described under observations. The variations studied in the external morphology of the control and experimental plants are described first, followed by their anatomical characteristics.

GENERAL MORPHOLOGY

Control plants grown for 12 weeks in the light condition had a prominent laterally elongated rhizome with a crozier. On an average on each plant about 12 leaves and 26 roots were formed (Figs. 1, 4, 24). The first leaf was small, had an elongated, flattened leaf blade, while the second leaf was obovate. The third and successive leaves had two leaflets. The leaflets were incompletely divided in 3rd to 5th leaf, while in the subsequent ones they were distinctly divided. The size and area of the leaflets increased in the successive leaves reaching the maximum size, with characteristic reniform shape from sixth leaf onwards. In the culture tubes the elongated petioles of 5th and subsequent leaves coiled around but the laminae were spread either near or on the surface of the liquid medium (Fig. 1). The roots were long, cream to brown in colour, and closely arranged on the lower side of the rhizome, with sparsely arranged root hairs on them. Axillary buds were formed on the lower part of the rhizome in the leaf axils, but they did not develop further.

Plants grown in the dark were etiolated. They had shorter, thinner, rhizomes and



FIGS. 1-11. *Regnellidium diphyllum.* Figs. 1-3. After 10 weeks of growth in liquid medium (under light). Fig. 1. control medium, Figs 2, 3. 2, 4-D medium 100 and 10 mg/L media (light) respectively, x 7/10. Figs. 4, 5. Plants in control medium grown in light and dark conditions respectively. Fig. 6. Plants in light from IAA 100 mg/L medium. Fig. 7. Part of the rhizome axis enlarged from one of them (surrounded by a circle). Fig. 8. Plant in dark IAA 100 mg/L medium. Fig. 9. Plants in light from IAA 1 mg/L medium. Notice the swollen part of the rhizome. Fig. 10. Plant in light from NAA 100 mg/L medium. Note the enlarged conditions of the rhizome and the abnormal leaves with reduced pinnae. **Fig. 11.** Rhizome portion enlarged to show several tissue outgrowths and many short roots.

less number of leaves with smaller leaflets (Figs. 5, 23, 24). Further in plants under dark conditions the leaves were parallelly arranged to the rhizome, oriented towards the rhizome apex in contrast to the plants under light, where the leaves were perpendicular to the rhizome (Figs. 4, 5). Roots were pale cream, short and few in number with few root hairs on them. The average number of leaves and roots formed in plants grown under light and dark conditions and the average length of the longest root and leaf are shown in Figs. 23, 24. Each figure represents the average value obtained from at least twenty measurements or plants. Lateral branches were absent in plants both in light and dark conditions.

EFFECTS OF IAA

At 100 mg/L concentration both under light and dark conditions, the rhizome elongation was inhibited and it grew as a swollen contorted structure (Figs. 6-8). The leaves and roots originated at irregular points around this massive structure and 10-12 root primordia were seen on the surface of swollen rhizome. Very close to the apical region 2-3 lateral branches originated (Fig. 7). The pinnae under dark conditions became variable with triangular, spoon or spatula shaped structures with wavy margins and some of them never attained mature form (Fig. 8).

At other concentrations, namely 10, 1 and .1 mg/L the rhizome apex was slightly enlarged only under light conditions (Fig. 9). The pinna size was reduced at higher concentrations but it reached almost the same size as in controls in plants grown in the .1 mg/L medium. The pinnae and roots were brown in colour showing toxic effects of IAA. The two higher concentrations used were more inhibitory for leaf production and leaf length than the lower two and the effects were similar both in plants under light and in dark (Fig. 23). Root numbers were greatly reduced (55-80%) at all concentrations in plants grown under light conditions, and under dark conditions the average number of roots produced per plant was less when compared with plants under light (Fig. 24). The number of roots formed in dark conditions in auxin media was slightly higher when compared with the control plants, and this was more so at lower concentrations (Fig. 24).

EFFECTS OF NAA

One week after incorporating NAA at 100 mg/L concentration, the rhizome apex lost its shape and became swollen. Further growth of such plants involved the enlargement of the rhizome into an amorphous structure, with a number of surface primordia. Some of these developed into massive bulges and others gave rise to a number of root and few leaf primordia (Figs. 10, 11). Such roots were short and arranged in close aggregates. The leaf primordia elongated into petioles which had either no pinnae or in certain cases the tips ended in delicately branched finger like structures with no resemblance to the pinnae of control plants (Fig. 10). Figure 11 is an enlargement of 10 showing the amorphous nature of the enlarged rhizome apex and root aggregates. The leaves and roots thus formed amidst the proliferated tissues were also irregularly arranged. Plants developed in dark showed some of these abnormal structures but they were less pronounced. The supernumerary short roots were not considered while determining the average number of roots.

Lateral branches were common.

Unlike the plants above described, at 10 mg /L concentration, both under light and dark conditions, the plants were bigger in size with more number of leaves and roots on them. All the leaves had distinct pinnae which were smaller in size. The roots were shorter, and brown in colour when compared with the controls. Both the leaves and roots were densely arranged on the rhizome.

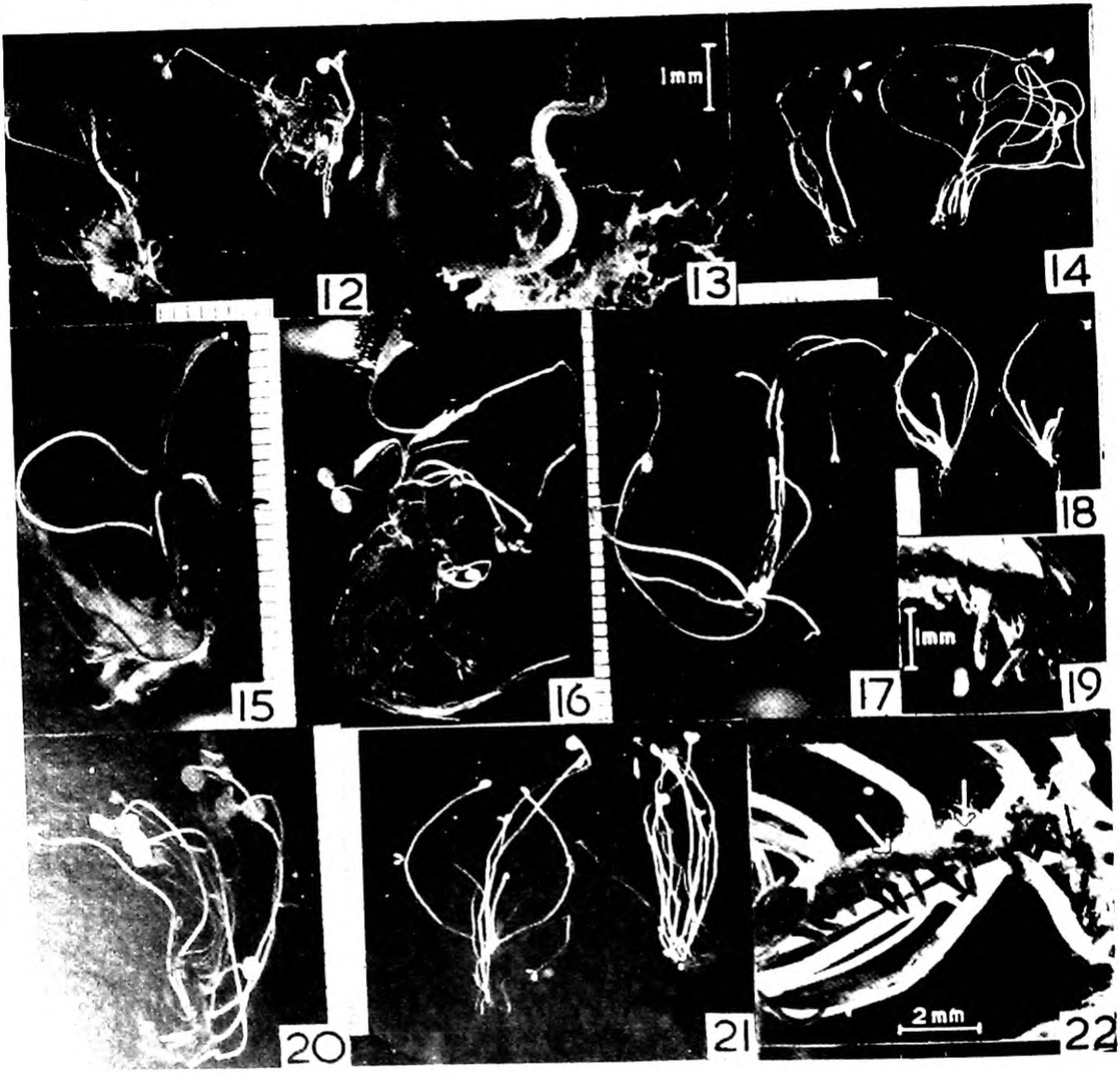
Plants developed in 1 mg/L media were smaller and those in .1 mg/L media resembled the plants of control group. The latter concentration was more favourable for leaf and root production both in light and dark conditions, showing an increase in their number over controls (Fig. 24). However, the average length of roots and leaves was almost like those of the controls. Lateral roots were present only in plants developed in 1 mg/L media. The rhizomes of plants grown under dark conditions were thinner, elongated and the leaves were closely arranged on either side of it.

EFFECTS OF 2, 4-D

At the time of incorporating 2, 4-D the sporelings had developed the first two leaves. Third leaf was partly developed and fourth one was in the primordial stage. At 100 mg/L concentration, one week after incorporation, the effects were obvious on the sporelings, both under light and dark conditions. A few weeks later the rhizome apices enlarged with many callus outgrowths and short roots (Fig. 2). These were densely covered with root hairs (Fig. 12). Only the third leaf developed with abnormal pinnae and the fourth leaf primordium merged with the rhizome callus. At the point where pinnae were attached the petiole was swollen. Plants in dark had rhizome callus and the root hairs were less dense, but more elongated (Fig. 13). The leaves also showed callus response where the pinnae were more swollen than the petioles with many outgrowths on them (Fig. 13). Even the pinnae that were formed earlier, before 2, 4-D was added, developed into swollen structures.

At 10 mg/L concentration, plants both in light and dark conditions showed similar abnormalities as in previous concentration (Fig. 15). In light the leaves were longer with elongated pinnae and in dark the pinnae were smaller. At 1 mg/L concentration the rhizome retained its normal shape but was slightly swollen. A number of lateral buds appeared and some of them developed into branches (Fig. 3). In plants growing in the dark the lateral buds were indistinct. In medium with .1 mg/L concentration the plants in light developed abnormal pinnae which were triangular or funnel shaped, and of bigger size. Plants grown in dark had elongated leaves, some only with small abortive pinnae (Figs. 16, 17).

Considerable variations were noticed in the number of roots formed, length of the longest root, number of lateral roots on them and the maximum number of laterals produced on any one root at a particular concentration and these are presented in Table 1. The concentrations 100 and 10 mg/L were inhibitory for root production and at other two concentrations the root length was slightly inhibited. Nearly 1/3 of the total number of roots formed had lateral roots on them in 1 and .1 mg/L media. The number of laterals on individual root was also more in these two media. Lateral roots were absent generally in plants grown in dark conditions (Figs. 16, 17). Branching and fasciation of roots were common at higher concent-



Figs. 12-22. [illegible] *diphyllum*. Figs. 12-13. Plants in 2, 4-D medium (100 mg/L) in light and dark conditions. Note the abundant root hairs, callus structures from rhizome and pinnae, abnormal pinnae. Fig. 14. Plants in GA (100 mg/L) medium under light conditions. Fig. 15. Plant from 2, 4-D (100 mg/L) medium under light. Note the abundant root hairs and abnormal pinnae. Figs. 16-17. Plants in 2, 4-D (1 mg/L) medium grown in light and dark conditions. Note abundant roots in 16, few roots in 17 and abnormal pinnae in both. Fig. 18. Plant in GA (100 mg/L) medium under dark. Fig. 19. A few roots enlarged (GA 10 mg/L-Light) to show their short length and kinked condition. Figs. 20-21. Plants in GA (1 mg/L) medium under light and dark conditions. Fig. 22. Part of the rhizome enlarged (GA 10 mg/L-Light) to show short roots and buds (arrows). Note the basal sheaths of the roots.

rations used and these abnormal structures are described in the following section. Root hair development was comparatively more in all the plants grown in media with different concentrations; the maximum condition seen in plants at 100 mg/L media (Figs. 2, 12). In the plants grown in dark, root hairs were either comparatively less or absent (Fig. 17).

TABLE I

SHOWING THE EFFECT OF 2, 4–D ON ROOT DEVELOPMENT IN *REGNELLIDIUM DIPHYLLUM*.

	Control medium	2,4—D			
		100	10	1	.1 mg/L
No. of roots	26*	4	8	38	36
Length of longest root	4.9 cm.	1.5	3.4	4.0	4.2
No. of roots with laterals	3	—	—	12	10
Maximum number of laterals on any one root	3	—	—	23	16

* Each figure represents the average of 20 counts or measurements.

EFFECTS OF GA

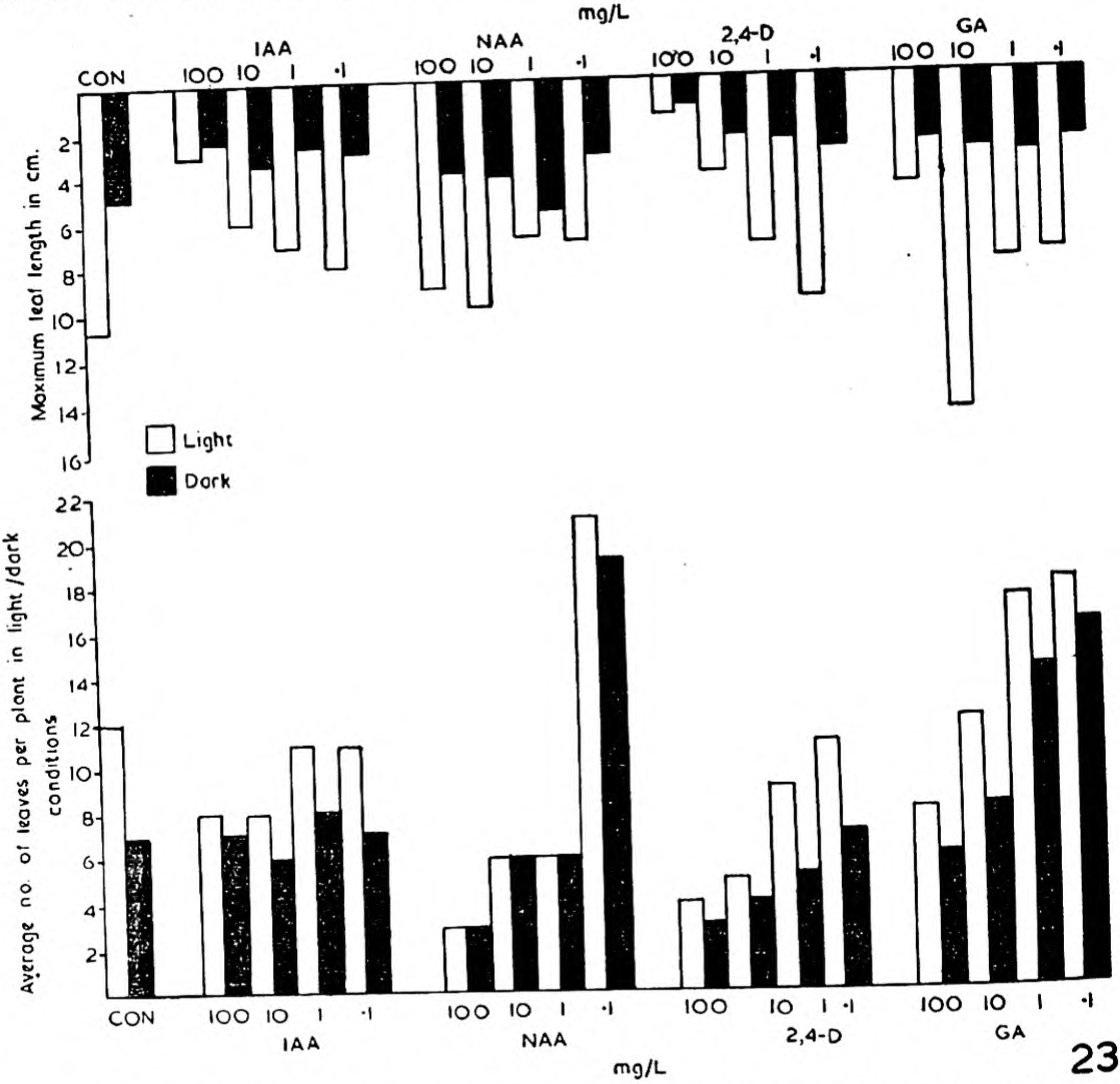
At 100 mg/L, the plants under light and dark conditions were smaller in size when compared with the controls (Figs. 14, 18). Less number of roots were formed than the leaves and growth was better under light conditions than in the dark (Figs. 23, 24). The rhizomes were condensed under both the conditions and crowded arrangement of roots and leaves was common. In GA media with 10- .1 mg/L concentration more roots were formed both in light and dark conditions (Fig. 24). But roots were short in all the plants both in light and dark conditions and thus GA was inhibitory for root elongation. The pinnae had normal shape but small in size in plants under light and they were very small or not enlarged under dark conditions (Fig. 14, 18). In few only leaf enations were present.

As compared with the controls the growth of plants was considerably improved in 10, 1 and .1 mg/L GA media and more number of leaves and roots were formed in the latter two media. Rhizomes were elongated with short internodes and was common for all the plants grown in light and dark conditions (Fig. 20, 21). The plants grown in dark were very fragile bleached in appearance, with filmy leaves. Pinnae were of normal shape but smaller in size. Lateral buds developed on the rhizomes of all the plants grown under light conditions except in media with 100 mg/L concentration (Fig. 22). Greater number of buds were formed, particularly at 1 mg/L concentration but of these only a few developed into lateral branches. The roots in all the plants were uniformly short, brown or dark brown in colour. In some the root tips were curled or kinky

(Fig. 19). Another important variation noticed was the absence of root hairs in all the concentrations. The epidermal tissue was swollen at the base of the roots and formed a black sheath (Fig. 19).

ANATOMICAL EFFECTS.

The anatomical details considered here mostly pertain to the rhizome, root and bud structures. Variations noticed in the plants treated with the growth substances will be comparatively considered with those of control plants.



23

FIG. 23. Graphic representation of average maximum leaf length and number of leaves produced in plants grown in different media under light or dark conditions. Each figure or measurement represents the average value obtained from 20 plants or leaves.

The transections of rhizomes, grown in control medium, showed one layered epidermis and 2-3 layered outer cortex.

Some epidermal cells developed into multicellular hairs or small glands. The inner portion of the cortex surrounding the stele consisted 3 or 4 layers of cells from the outer region of which the partitions radiated. The amphiphloic siphonostele and pith regions were well differentiated (Fig. 25). The root as seen in cross section showed a distinct enlarged piliferous layer surrounding 2 or 3 cortical layers. The septae radiated from the small inner cortex and the stele showed diarch condition (Fig. 32). The longitudinal sections of rhizomes showed the position of undeveloped buds (Fig. 30).

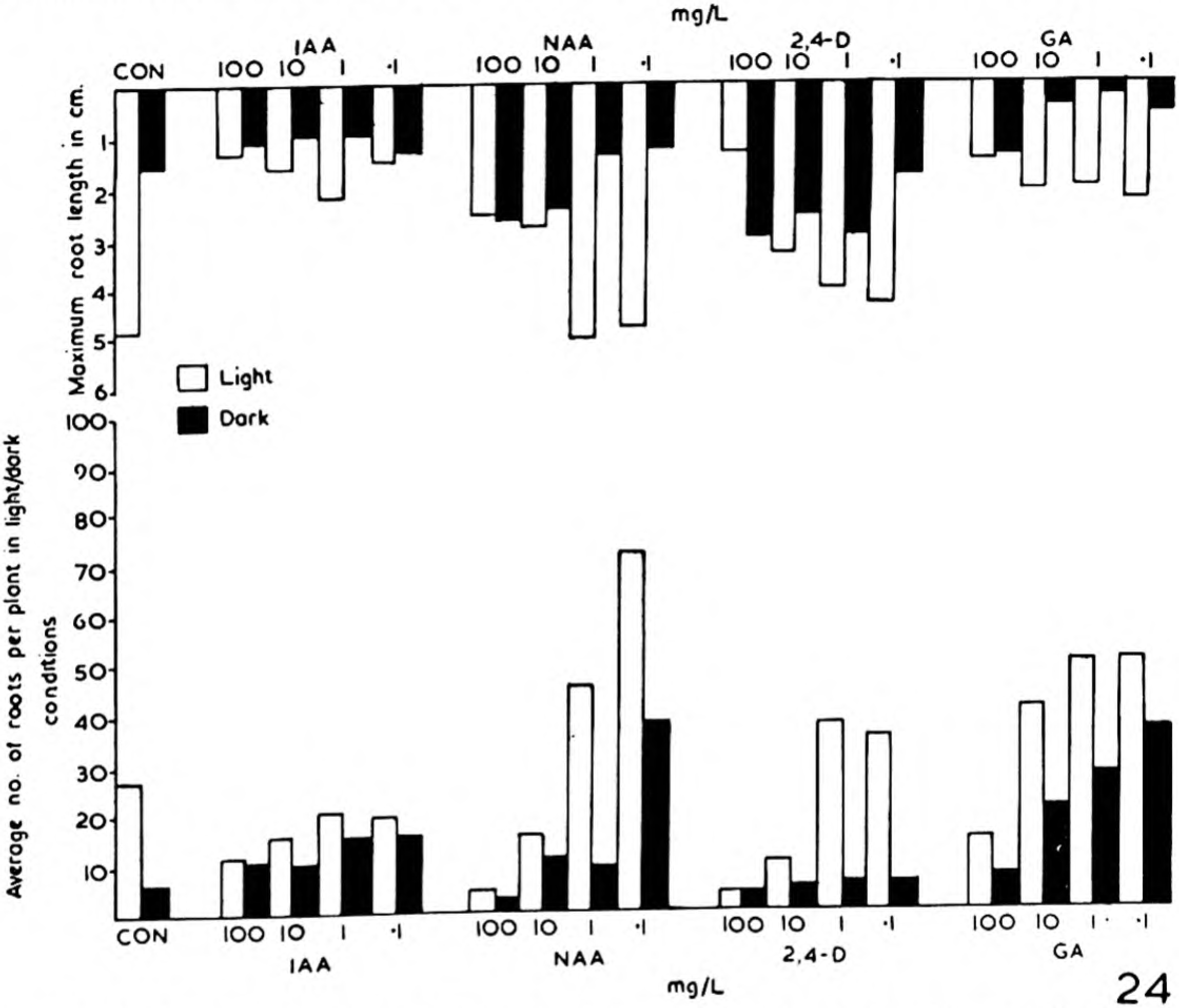
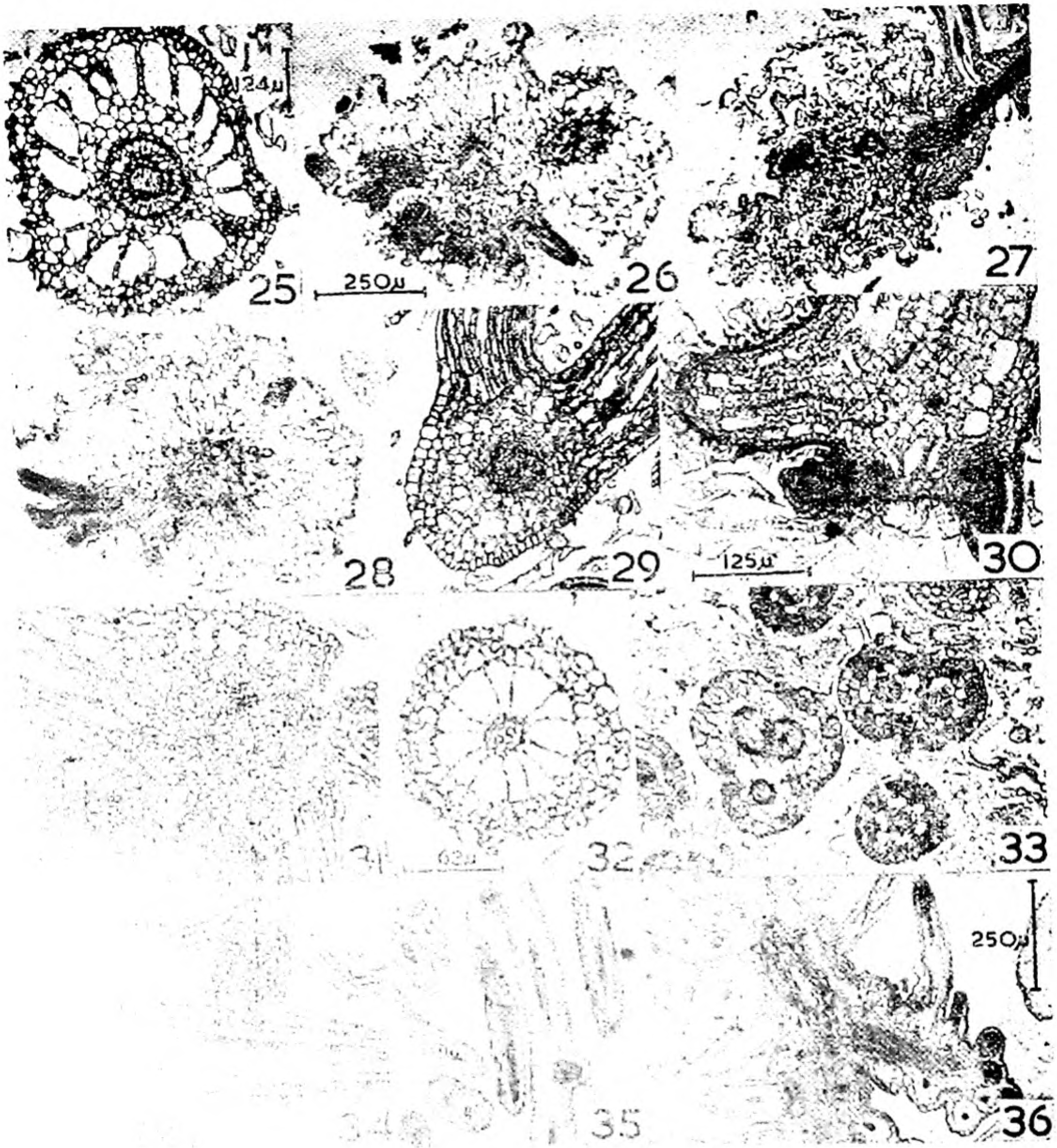


FIG. 24. Graphic representation of average maximum root length and number of roots produced in plants grown in different media under light or dark conditions. Each figure or measurement represents the average value obtained from 20 plants or roots.

At 100 mg/L concentration the rhizomes of plants (except in GA medium) were contorted and swollen. The transverse sections of such rhizomes, developed in IAA and NAA media, showed the enlarged epidermal cells and outer cortical tissue that developed into distinct proliferations. Both cortical and stelar tissues were irregularly arranged with no air spaces. The abnormalities were more intense in rhizomes of 2, 4-D medium. Figure 26 shows the



[illegible] izome of control plant. Figs. 26. T. s. callus of rhizome, out- [illegible] from inner cortex. Fig. 27. L. s. callus in terminal region of [illegible] Fig. 28, with uneven outline of rhizome callus with many out- [illegible] L. s. callus of rhizome showing the origin of fasciated root. 26-28, [illegible] of IAA medium (1 mg/L. Light). Fig. 30. L. s. rhizome of [illegible] leaf axil covered with hairs and L. s. root primordium. Fig. [illegible] (1 mg/L. light) nodal region showing 2 leaves, a root and a lateral [illegible] 32. T. s. root control plants, light. Fig. 33. T. s. of two fasciated and [illegible] are present in the fasciated structures. Fig. 34. L. s. root (control) [illegible] through the cortex. 33, 34. Common scale. Fig. 35. L. s. [illegible] 0 mg/L, dark) showing fasciated condition and development of laterals. [illegible] plant in 2, 4-D medium (1 mg/L, light) showing many roots and leaves [illegible] uneven outline of the rhizome with many protrubernances formed by epidermal and cortical tissues. The cortical cells were much enlarged, arranged in regular rows, without any air spaces between them. The stelar region is pushed to a side, seen on the right side of the picture, due to the overgrowth of a rhizome portion. In the enlarged portion many root primordia developed, some near the peripheral region and others in the centre of the newly formed tissues. Figure 27 is a longitudinal section of the abnormal outgrowth formed in the terminal part of the rhizome showing the proliferated callus tissues with many outgrowths. These outgrowths had very loosely arranged groups of cells and in certain cases isolated cells were separated from the callus outgrowths. The dark central strand is the stelar region with less darker but compactly arranged tissues on either side of it that represent cortex. In GA medium, the proliferation of tissue was absent.

Some of these abnormalities were also seen, but less evidently, in rhizomes developed in media with different growth substances at 10 mg/L concentration. At lower concentrations (1 and .1 mg/L) the epidermis was intact, the cortical cells were compactly arranged and the air spaces were indistinct or sometimes completely obliterated (Fig. 29). Since the internodes were short, many root or leaf primordia originated in any one nodal region (Figs. 29, 31).

Very few buds developed on the control plants in the nodal region located between the leaf and the root as shown in Fig. 30. These buds remained dormant and were covered with many multicellular epidermal hairs. The apex of the bud was conical or dome shaped with a characteristic apical cell and the subdistal region. The buds were more frequently formed on rhizomes developed in media with lower concentrations (1 and .1 mg/L) of IAA, 2, 4-D and GA. Their position was irregular, developed both on the upper and lower portion of the rhizome, and again sometimes in juxtaposition to roots or leaves. Of these, greater number of buds, were arranged irregularly and were common in GA media. The apex had varied outline in some of them (Figs. 30, 31). Many buds developed into lateral branches with several roots and few leaves on them. Numerous branches of this type were seen in 2, 4-D, NAA, IAA media, and one of these is illustrated in Fig. 36 with 2-3 leaves and about eight roots.

Roots formed in the control medium showed distinct epidermis, 3-4 layered cortex that surrounded the 10-12 air canals (Fig. 32). Some of the epidermal cells were enlarged. The inner cortex was limited that surrounded the central stelar region (Fig. 32). Extreme abnormalities were noticed in the root structure, in size, number of roots involved and tissue arrangement. At higher concentrations, at 100 and 10 mg/L, the cortical tissues were ill developed with few or no air spaces (Fig. 28). Fasciated roots developed mostly in 2, 4-D media and occasionally in GA and NAA media at 100 mg/L concentration. Such roots were short and stumpy and they arose from the inner cortical region of the rhizome (Figs. 26, 28). In 2, 4-D and NAA media this region consisted of callus or proliferated tissue with compactly or loosely arranged cells while in GA media they were adjacent to the stelar reigon. From a common primordium two or three roots originated and in their subsequent development either they were completely separated from each other or remained fused (Figs. 26, 28). Transverse sections of these roots showed a common cortex and 2-3 steles were visible within each one of them (Figs. 33, 35). The wavy outline indicated the number of roots fused together and the meristeles were also fused or remained separate from each other (Fig. 33). The epidermis was distinct with two layered cortex and most of the cells developed tannin contents in them. The vascular tissues in the stele were scanty.

At lower concentrations the roots were smaller in their cross sectional area but the tissue arrangement resembled those of control roots.

Another interesting variation noticed was regarding the development of lateral roots that originated from the pericyclic region. Figure 34 shows the control roots with a lateral root primordium. In media with 1 and .1 mg/L concentrations many primordia were seen in the short roots, some of them developed into short lateral roots and others were aborted (Figs, 24, 35). The number of laterals decreased along the concentration of the different growth substances.

In such plants where the root hairs developed in great abundance the root epidermal cells were very much enlarged, and in others the hair producing cells were of the same size as the non-root hair cells.

DISCUSSION

The morphogenetic effects of sugars were emphasized in the earlier papers and inclusion of sugar in the medium was essential for the growth of this plant under invitro conditions (Rao, 1966a, b). A higher concentration of glucose or sucrose was required for good growth of *Marsilea* or *Salvinia* when compared with *Regnellidium and Salvinia* did not grow well in glucose medium (Allsopp, 1953; Gaudet and Koh, 1968). Both in light and dark conditions the sporelings could grow but the growth was much better in cultures under light. The growth requirements of the aquatic pteridophytes are similar to *Utricularia* and other flowering plants (Pringsheim and Pringsheim 1962; Mohan Ram et al, 1971).

The physiological effectiveness of the growh substances on *Regnellidium* is in accordance with the generalisations made earlier (Thomson,1945; Gautheret, 1945, Allsopp, 1952). The concentration effects of these substances were variable. Comparatively IAA, at all concentrations, was more inhibitory for root production and root elongation. At higher concentrations NAA, 2, 4-D and GA were inhibitory for root production. At 100-mg/L concentration GA was inhibitory for root production in *Marsilea* (Allsopp, 1962). The inhibitory influence of growth substances was less marked on leaf numbers and root length. In media with lower concentrations of NAA and GA more number of leaves were formed than in control plants. Root length increased in plants that developed in lower concentrations of NAA and 2, 4-D media. The inhibitory influence of higher concentrations of IAA, NAA, and 2, 4-D on the growth of *Marsilea* plants are recorded (Allsopp, 1952, 1963). Dark condition was generally inhibitory for leaf and pinnae development. In many instances the pinnae were abortive and did not progress beyond the primordial stage; though petiole elongation was not inhibited. Abnormal pinnae were formed in 2, 4-D media and these were different from the controls both in size and shape. In *Marsilea* plants, under the influence of 2, 4-D, not only the number of leaves were reduced but they were also juvenile in nature. Crowding of roots and formation of abundant lateral roots and root hairs were seen in 2, 4-D media and such marked developmental changes were similar to those recorded in *Marsilea* (Allsopp,1952). In *Marsilea* plants lateral roots were formed in IAA medium (10^{-6}) but in *Regnellidium* such a response was not seen at any concentration of IAA media (Allsopp, 1952). The metabolic changes in auxin-protein relationship of the tissues and the influence of auxins in induction of roots are discussed earlier (Skoog, 1951). Distinct root tumours were formed in *Marsilea* but they were absent in *Regnellidium* plants (Allsopp 1952). The growth substances were effective on weight increase in *Salvinia* but the anatomical characters of these plants are not given (Gaudet and Koh, 1968).

Higher concentrations of IAA, NAA and 2, 4-D effected the growth of rhizome apex which responded and grew into enlarged massive structure. Under the influence of IAA and NAA the cortical tissues were loosely arranged and developed into proliferations. Compactly arranged callus tissue development was common in 2, 4-D media. In such cases the tissue building materials were diverged from the organ systems to callus tissue development. Proliferation of tissues was common in auxinic cultures of *Marsilea* but not callus formation (Allsopp, 1952). Complete or partial absence of air spaces in the cortex, indistinction between cortical and stelar regions were seen in rhizomes developed in different media. Such abnormalities were less marked or totally absent in media with lower concentrations of growth substances.

Another interesting variation noticed was with regard to the development of axillary buds. In control plants bud initials were formed in the leaf axils but rarely they developed into branches. But in IAA, NAA, 2,4-D and GA media many of them not only developed into branches but also they showed many abnormalities in their internal structures. Positional variations in bud development were seen in rhizomes developed in GA media. The non development of buds into lateral branches in control plants indicated the inhibitory influence present on them and the inhibition was removed by the presence of the growth substances. The pronounced apical dominance, the nutritional status of the parent axis and the influence of IAA and IAN on bud development were discussed in *Marsilea* plants (Allsopp, 1963).

Lateral fusion of different structures in natural self-grafting is considered as one aspect of fasciation. Fasciated roots observed in the present study confined to the above description. Various factors, both external and internal, were found to produce fasciation among which high concentrations of growth substances are included (Sinnott, 1960; Bloch, 1938). Fasciated roots were observed in *Marsilea* cultures grown under in vitro conditions (Allsopp, 1952).

Most of this work was done in Department of Crptogamic Botany, University of Manchester during 1967-68. This paper is dedicated in memory of late Dr. A. Allsopp, with whom the author had the opportunity to work and discuss many of the observations recorded here. I am grateful to Professors C. W. Wardlaw, J. Colhoun, and E. G. Cutter for their help and interest. My thanks are due to the executives of Royal Society, London, for the award of a visiting Research Fellowship. I am indebted to Professor Robert L. Hulbary, State University of Iowa, Iowa, City, U.S.A., for his encouragement and sending the sporocarps used in this study.

REFERENCES

Allsopp, A. 1952. Experimental and analytical studies of pteridophytes. XVII. The effect of various physiologically active substances on the development of *Marsilea* in sterile culture. *Ann. Bot. N. S.* **16:** 165-183.

——. 1953 Experimental and analytical studies of pteridophytes. XIX. Investigations on *Marsilea*. 2. Induced reversion to juvenile Stages. *Ann. Bot. N. S.* **17:** 38-55.

——. 1962. The effects of gibberellic acid on morphogenesis in *Marsilea drummondi* A. Br. *Phytomorphology*. **12:** 1-10.

——. 1963. Morphogenesis in *Marsilea*. *J. Linn. Soc., Bot.* **58:** 417-427.

——. 1964. The metabolic status and morphogenesis. *Phytomorphology* **14:** 1-27.

Bloch, R. 1938. Anatomical changes in *Tradescantia fluminensis*. Vell after treatment with growth substances. *Contrib. Boyce Thompson Inst.* **9:** 439-454.

Gaudet, J. J. 1964. Morphology of *Marsilea vestita*. I. Ontogeny and morphology of the submerged and land forms of juvenile leaves. *Am. J. Bot.* **51:** 495-502.

——. 1964a. Morphology of *Marsilea vestita*. II. Morphology of the adult land and submerged leaves. *Am. J. Bot.* **51:** 591-597.

——. 1965. Morphology of *Marsilea vestita*. III. Morphogenesis of the leaves of etiolated plants. *Am. J. Bot.* **52:** 716-719.

——. 1965a. The effects of various environmental factors on the leaf form of the aquatic fern *Marsilea vestita*. *Physiologia Pl.* **18:** 674-686.

——. 1967. The grwoth of excised leaves of *Marsilea*. *Can. J. Bot.* **45:** 1127-1134.

——, and D. V. Koh. 1968. Effect of various growth regulators on *Salvinia rotundifolia*. *Bull. Torrey bot. Cl.* **95:** 92-102.

Gautheret, R. J. 1945. *La Culture des Tissus.* Mason and Cie., Paris.

Mohan Ram, H. Y. , H. Harada, and J. P. Nitsch. 1971. Effect of some growth substances and inhibitors on the growth and flowering of *Utricularia inflexa* in vitro. In *Plant Cell, Tissue and Organ Cultures.* Intl. Symp. on Morphogenesis. University of Delhi. 32-33.

Ntsch. J. P. 1951. Growth and development in vitro of excised ovaries. *Am. J. Bot.* **38:** 566-577.

Pringsheim, E. G., and O. Pringsheim. 1962. Axenic culture of *Utricularia*. *Am. J. Bot.* **49:** 898-901.

——, and ——. 1967. Kleiner Beitrag zur Physiologie von *Utricularia*. *Z. Pflanzen-Physiol.* **57:** 1-10.

Rao, A. N. 1966a. Morphogenetic effects of sugars on *Regnellidium diphyllum* Lind. *Flora* **156:** 378-387.

——. 1966b. Morphologenetic effects of sugars and mannitol on *Regnellidium diphyllum*. *Flora* **156:** 452-463.

Sinnott, E. W. 1960. *Plant Morphogenesis.* McGraw-Hill, New York.

Skoog, F. (ed). 1951. *Plant Growth Substances.* University of Wisconsin Press, Madison.

Thomson, B. F. 1945. Tissue responses to physiologically acitive substances. *Bot. Rev.* **11:** 593-610.

White, R.A. 1966. The morphological effects of protein synthesis inhibition in *Marsilea*. *Am. J. Bot.* **53:** 158-165.

——. 1968. A correlation between the apical cell and the heteroblastic leaf series in *Marsilea*. *Am. J. Bot.* **55:** 485-493.

Adv. Pl. Morph. 1972: 339–358

IN VITRO GROWTH RESPONSES OF MATURE ENDOSPERM OF *RICINUS COMMUNIS* L.

By B. M. Johri and P. S. Srivastava[1]

Department of Botany, University of Delhi, Delhi 7, India

Abstract

Endosperm is an excellent experimental system for physiological and morphogenic studies. Growth responses of mature endosperm of *Ricinus communis* are reported in this paper.

Initially, endosperm with the embryo intact was cultured on WB. The embryo formed a normal seedling. Addition of an auxin did not alter the pattern of germination of the embryo. However, 2, 4-D added to WB inhibited the growth of radicle and delayed germination; the endosperm also showed some activity. On WB + a cytokinin, there was no visible morphological change in endosperm. A combination of WB + an auxin + a cytokinin also did not result in any significant response. When WB + an auxin + a cytokinin was fortified with either CH or YE, there was profuse proliferation of the mature endosperm tissue. The endosperm showed satisfactory growth on WB + 2, 4-D + KN + YE.

The studies on growth response of mature endosperm of *Ricinus* revealed that it grows satisfactorily at *p*H 5.0, with 4% sucrose. Besides sucrose, IAA, KN, CH and YE, growth response was also tested using other sugars, auxins and cytokinins. While 4% sucrose proved most satisfactory (1440% fresh weight) for growth, glucose and maltose were also effective; the least effective was mannose (145% fresh weight increase). Amongst phenoxyacetic acids (10^{-5} M), 2, 4-D proved most effective (1360% increase in fresh weight) and 2, 4, 5-T the least. Of the substituted aminopurines tested, SD 8339 brought about maximum growth (1425% increase in fresh weight); minimum growth (565% fresh weight increase) occurred with triacanthine. On 3000 ppm YE there was 1425% fresh weight increase and 385% increase in dry weight; 2,500 ppm YE resulted in 1395% fresh weight and 421% dry weight increase. After continuous growth on WB + 2, 4-D + KN + YE for 11 months, the callus could be maintained on WB + CH. To obtain maximum growth of the tissue, 8 different concentrations of CH were tried. Of these, 2,000 ppm was most effective. Attempts to replace the effect of CH on callus growth either by a mixture of amino acids, or by individual amino acids, revealed that it was possible to do so to some extent, by using a mixture of 12 amino acids, or a single amino acid (glutamic acid at 1.4×10^{-5}M).

The cells of endosperm callus were of diverse shape and size; differentiation of tracheid-like cells was very common in 8 to 10-week-old cultures; and cell aggregates which simulated stages of embryogeny were also discernible in 12-week-old cultures. Anatomical studies of compact callus showed zonal differentiation of vascular tissue simulating vascular bundles. Organogenesis was not observed.

---

[1] Present Address: Department of Botany, S. G. T. B. Khalsa College, New Delhi 5.

INTRODUCTION

The fact that one of the male gametes participates in triple fusion has led to much discussion as to the morphological nature of the endosperm tissue (see Maheshwari, 1950). This has often been called a 'maimed embryo'. Its main function is to store reserve food to be utilized by the developing embryo. That the endosperm plays an important role in embryonic growth has been emphasized by various workers (Sargant, 1900; Van Overbeek et al., 1942; Brink and Cooper, 1947; see also Raghavan, 1966).

While in some instances the endosperm tissue is consumed during seed development, in others it persists even in the mature seed and is used up during the initial stages of embryo germination.

Being one of the products of double fertilization, the limited growth of endosperm has always been questioned. Furthermore, its inability for organizing into a plant, unlike the embryo, has always been intriguing. The reason for this incapability of the endosperm can not be its triploid nature, as diploid endosperm does occur in angiosperms.

Since the report of Haberlandt (1902), who stated that the result of culture experiments should give some interesting insight to the properties and potentialities which the cell as a preliminary organism possesses, the technique of cultivating isolated plant cells in nutrient medium has led to the investigations of important problems of morphogenesis (see Krikorian and Berquam, 1969).

Various attempts have been made to culture immature (LaRue, 1949; Lampton, 1952; Norstog, 1956; Nakajima, 1962; Tabata and Motoyoshi, 1965) and mature (Rangaswamy and Rao, 1963; Johri and Bhojwani, 1965; Bhojwani, 1968; Nag, 1970) endosperm of several taxa to answer some of the questions mentioned in the foregoing paragraphs.

A survey of the literature revealed that there is enough data on the growth and morphogenesis of diploid tissues, but scant attention has been paid to understand the nutritional requirements of triploid tissue (see Straus and La Rue, 1954; Nakajima, 1962). The present investigation was, therefore, undertaken to study the growth requirements of mature endosperm.

MATERIAL AND METHODS

Ricinus communis L. was selected as the experimental material. It is tall, glabrous, erect annual herb which, sometimes, becomes shrubby or even tree-like . The fruits are schizocarpic capsules dehiscing into three cocci known as 'regma'. The seeds are characteristically marked, and the endosperm contains 35 to 55% thick, colourless or greenish oil.

Fresh, ripe fruits of *R. communis* were collected from the Dehli University campus. These were washed thoroughly with tap water, and then with rectified spirit. The hard seed coat was removed, and the endosperm with the embryo intact was excised under sterile conditions. This was washed with 0·2% solution of cetrimide (commercially sold as 'Cetavlon' by ICI), and treated with 90% ethyl alcohol for about 4 minutes. Before inoculation, the explants were washed throughly with sterile distilled water.

A modified White's medium (see Rangaswamy, 1961), henceforth referred to as WB, was used. One litre of the medium contained :

A. *Macro- elements*	mg/1
$MgSO_4.7H_2O$	360.00
$Ca(NO_3)_2.4H_2O$	275.00

Na_2SO_4	200.00
$NaH_2PO_4 . 2H_2O$	175.00
KNO_3	80.00
KCl	65.00
B. *Micro-elements*	
$MnSO_4 . H_2O$	3.00
$ZnSO_4 . 7H_2O$	0.50
$CuSO_4 . 7H_2O$	0.025
$CoCl_2 . 6H_2O$	0.025
$Na_2MoO_4 . 2H_2O$	0.025
H_3BO_3	0.50
C. *Iron-source*	
$Fe(C_6H_5O_7) \cdot 3H_2O$	10.00
D. *Amino acids and Vitamins*	
Glycine	7.50
Niacin	1.25
Thiamine hydrochloride	0.25
Pyridoxine hydrochloride	0.25
Calcium pantothenate	0.25

Various other salts, in addition to, or in place of, those recommended by White (1963), were incorporated in the medium to find out the best medium for the growth of endosperm, as well as the callus, derived therefrom.

Normally, sucrose was used as the sole carbon source but, in order to determine the most suitable carbon source, it was replaced by arabinose, fructose, galactose, glucose, mannose, sorbose, cellobiose, lactose, maltose, and raffinose. In several treatments, the following supplements were added, individually or in various combinations, before autoclaving the medium :

Indole-3-acetic acid (IAA); indole-3-butyric acid (IBA); indole-3-propionic acid (IPA); 2, 4-dichlorophenoxyacetic acid (2, 4-D); 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T); O-phenoxyacetic acid (O-PAA); p-phenoxyacetic acid (p-PAA); naphthaleneacetic acid (NAA); adenine (Ad); benzyladenine (BA); 6-(benzylamino)-9-(tetrahydropyran-2-yl)-purine (SD 8339) ; 3-(γ, γ-dimethylallylamino)-purine (triacanthine); 6-(γ, γ-dimetheylallylamino)-purine (6-γ, γ); 6-(4-hydroxy 3-methylbut-trans-2-enyl)-aminopurin-(zeatin); 1, 3-diphenylurea (DPU); casein hydrolysate (CH); yeast extract (YE).

Analytical grade chemicals, and Pyrex glass-distilled water were used for the preparation of media. The medium was jelled with 0.8% Difco Bacto-agar; *p*H of the medium (unless otherwise mentioned) was adjusted before autoclaving at 5.6, using 0.1N NaOH or HCl, with Beckman glass-electrode *p*H meter. Twenty ml of hot medium was dispensed into Pyrex or Corning culture tubes. After plugging with non-absorbent cotton wrapped in cheese cloth, the tubes were autoclaved at 1.06 Kg/cm^2.

Each experiment was usually repeated three times, with atleast 24 tubes in each treatment. The cultures were maintained under diffuse-day-light (50-100 lux) at 25±2C and 50-60% relative humidity.

The cultures of mature endosperm, without the intact embryo, did not show any response. Therefore, the embryo was usually left intact in the explants. However, soon after the initiation of proliferation of endosperm, the embryo was removed.

The percentage of cultures giving positive or negative response was taken into consideration for evaluating the effects of various treatments. For squash preparations either the fresh tissue was used or that fixed in acetic-alcohol (1:3, glacial acetic acid : absolute alcohol) for 24 hrs and washed and stored in 70% alcohol. Squash preparations were stained with 1% acetocarmine, made permanent after dehydration, and mounted in canada balsam. The customary procedures for dehydration in ethyl alcohol-xylol series and embedding in paraffin-wax were followed for histological studies. The material was sectioned at 10 to 15μ, stained either with safranin-fast green or haematoxylin-orange G, and mounted in canada balsam.

Experiments were conducted by selecting actively-growing regions of tissue in stock cultures. Generally, 125 mg of tissue was obtained from several cultures. At the end of an experiment, which lasted 6 weeks, the tissue was removed from culture vials, gently soaked with blotting paper to remove as much excess water as possible and as much agar as practicable, and the total weight of the pooled tissue was determined.

Fresh weight was determined along with dry weight, since the difference between the two seems to throw light on the growth response of the tissue under different experimental conditions. The percentage change in dry weight was determined by drying the tissue in an oven, maintained at 60±4C, till the weight became constant, and weighed on a Mettler balance.

Lignin deposition was tested with phloroglucinol (Jensen, 1962). In the proliferating callus mass, the tests were performed either in squash preparations of the fresh tissue, or in free hand sections of the compact endosperm callus.

RESULTS

On WB, the intact embryo (enclosed in endosperm) (Fig. 1A, B, C) remained quiescent for 1 week. The radicle emerged after 7 days; this was accompanied by bursting of endosperm all over its surface. Within another week the cotyledons turned green, and a normal seedling was established after 5 weeks. The endoperm is consumed by the developing seedling within 7 weeks (Fig. 1D). The increase in sucrose concentration in the medium did not alter the response.

IAA, IBA, IPA, or NAA (0.5, 1.0, 2.0, 2.5, 4.0 and 5.0 ppm), added to WB, did not induce proliferation. With 1.0 to 2.0 ppm, the endosperm exhibited slight swelling and elongation after 10 days; at higher concentrations the endosperm shrivelled. A normal seedling was obtained with all the auxins except 2, 4-D. WB+IBA (4.0 and 8.0 ppm) inhibited the growth of radicle which swelled and showed reversed polarity.

WB+2, 4-D gave a better response and, while the endosperm proliferated, germination of embryo was delayed. The radicle also swelled and proliferated in 2-week-old cultures. The proliferated endosperm failed to grow in-subcultures. WB containing GA_3 (1.0 to 5.0 ppm) gave a more or less similar response to that on WB+IAA or IPA, except that the embryo germinated early. On WB+KN (1.0 to 5.0 ppm) the endosperm swelled slightly. WB+KN (2.0 ppm) delayed germination, and inhibited the growth of radicle. There was slight proliferation of endosperm, after 4 weeks on WB+KN (5.0 ppm).

Nonetheless, a combination of an auxin and cytokinin (KN) gave better results. WB supplemented with lower concentrations of IAA, IBA, IPA or NAA+KN, did not show any visible morphological change in the mature endosperm tissue. However, on WB+NAA (2.0 ppm)+KN (5.0 ppm), the two halves of the endosperm, after initiation of germination of embryo, showed some proliferation from the margins, within two weeks. The callus tissue became nodulated after 5 weeks turned brown, and could not be maintained. With WB+2, 4-D (2.0 ppm)+KN (5.0 ppm), though the germination of embryo was suppressed, the endosperm proliferated. The friable

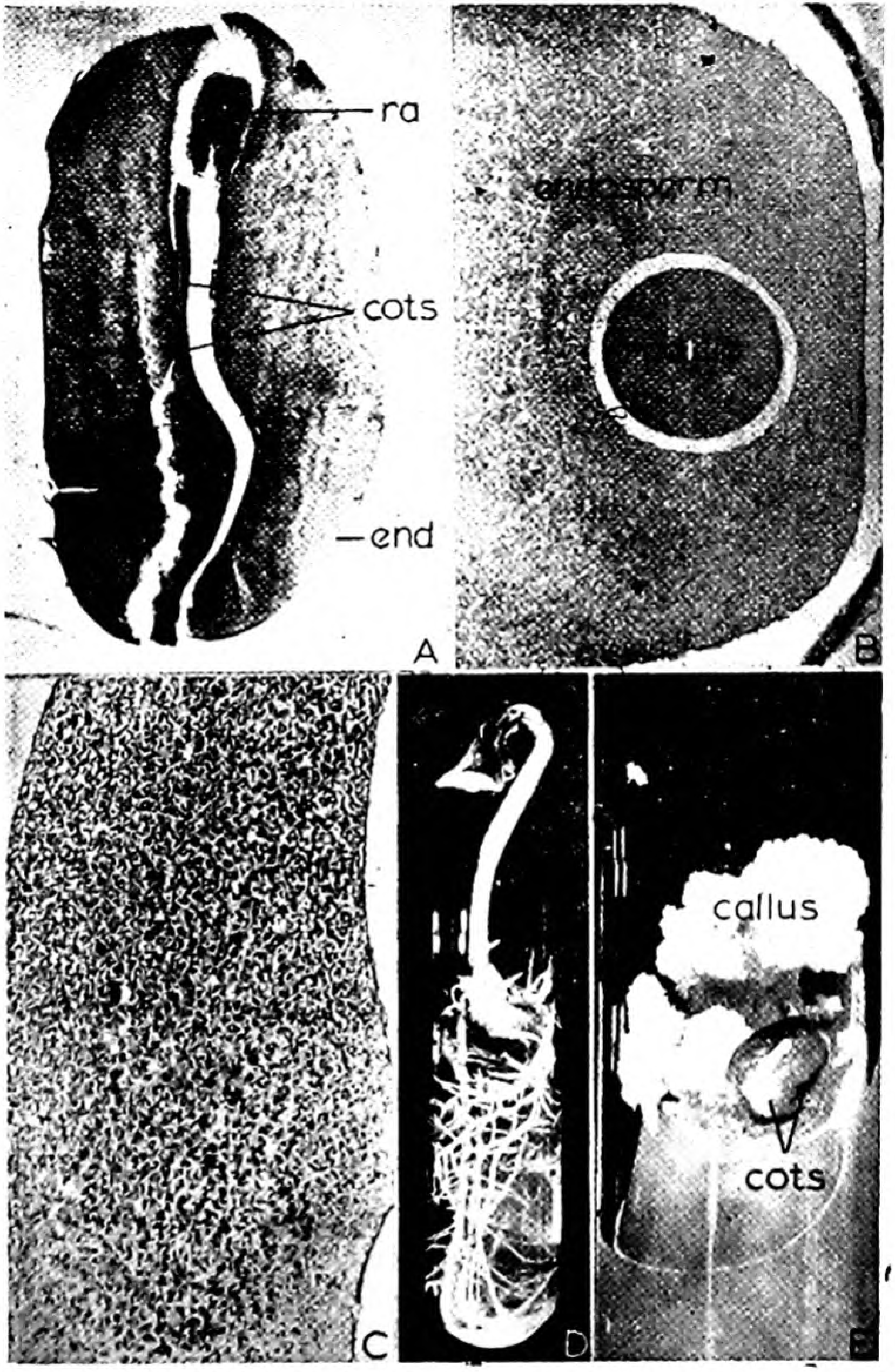


FIG. 1 A-E. A. Longisection of embryo with endosperm; bulk of the seed is occupied by endosperm, x 13. B. Transection at radicular end of decoated seed, x 30. C. Endosperm cells with abundant lipid contents, x 26. D. 7-week-old seedling, on WB; the entire endosperm has been consumed, x 1. E. 8-week-old endosperm callus, on WB + 2,4-D (2.0 ppm) + KN (5.0 ppm) + YE (2500 ppm); the cotyledons have failed to proliferate, x 1.75.

ra, radicle; *cots*, cotyledons; *end*, endosperm.

callus, after 4 subcultures, each passage of 25 days, turned brown and collapsed.

The addition of CM to WB resulted in a robust seedling. On WB+CM (10%) the endosperm swelled considerably, after 2 weeks, in 22% cultures. After 4 weeks the endosperm increased in size and split open into two halves; 32% 6-week-old cultures showed proliferation. Higher concentrations of CM did not bring about any appreciable change than those at lower concentrations.

WB+CH proved better than WB+CM. On WB+CH (500 ppm), germination of the embryo was delayed; CH (1000 to 2000 ppm) brought about further inhibition in germination. The endosperm showed some swelling and cracks all over its surface, but failed to form callus. A more or less similar response was observed with WB+YE. Like WB+CH, WB+YE (500, 1000 ppm) also delayed germination of the embryo. Even with 2000, 2500 and 3000 ppm YE the endosperm did not show any significant proliferation.

[illegible] combination of auxin and CM, CH [illegible] IAA, IBA, IPA, or NAA [illegible] YE) also did not bring [illegible] significant change in the [illegible] endosperm tissue. WB+2,4-D [illegible] resulted in profuse callusing [illegible] cultures. However, this [illegible] be maintained in sub-[illegible] of KN to WB+YE [illegible] ation in 41% cul-[illegible] proliferation of [illegible] 31% cultures. [illegible] failed to grow [illegible] medium.

Several trials [illegible] formulate a medium suitable for the growth and proliferation of mature endosperm. Since none of the above-mentioned media proved satisfactory, in further experiments WB was supplemented with an auxin, a cytokinin, and CM, CH, or YE.

The endosperm tissue showed significant response on WB+IAA (2.0 ppm) +KN (5.0 ppm)+CH (1000 ppm) and on WB+2,4-D (2.0 ppm)+KN (5.0 ppm) +YE (2500 ppm). WB+2,4-D (2.0 ppm) +KN (5.0 ppm)+CH (1000 ppm) induced proliferation in 10-day-old cultures, but did not prove as satisfactory as WB+2,4-D (2.0 ppm)+KN (5.0 ppm) +YE (2500 ppm) (Fig. 1E). On the latter medium, the growth of the radicle was completely suppressed and the embryo did not form a normal seedling. The endosperm proliferated profusely and produced a subculturable mass of tissue, after 6 weeks, in 56% cultures. In subcultures the endosperm callus showed satisfactory growth.

To begin with, the callus was fragile and white. Later, it became compact (Fig. 2A) upon continuous subculture for 4 months, each passage of 30 days. The callus turned yellow and then brown after 10 weeks.

GROWTH RESPONSE

The actively-growing callus, on WB+2,4-D+KN+YE was cut into smaller pieces, and each piece planted on a fresh medium. Thus, several clones were developed which were used for further experimentation.

An experiment was set up to study the effect of *p*H of the medium on the growth of callus. The results are summarised in Table I.

The maximum growth of endosperm callus occurred at *p*H 5.0 with 1320% increase in fresh, and 350% increase in dry weight. There was a sudden increase in the growth rate in *p*H from 4.5 to 5.0 (Fig. 3).

In the next set of experiments an attempt was made to determine a suitable concentration of sucrose for the best growth of callus. Various concentrations of sucrose were added to WB+2,4-D+KN +YE. The results are given in Table II.

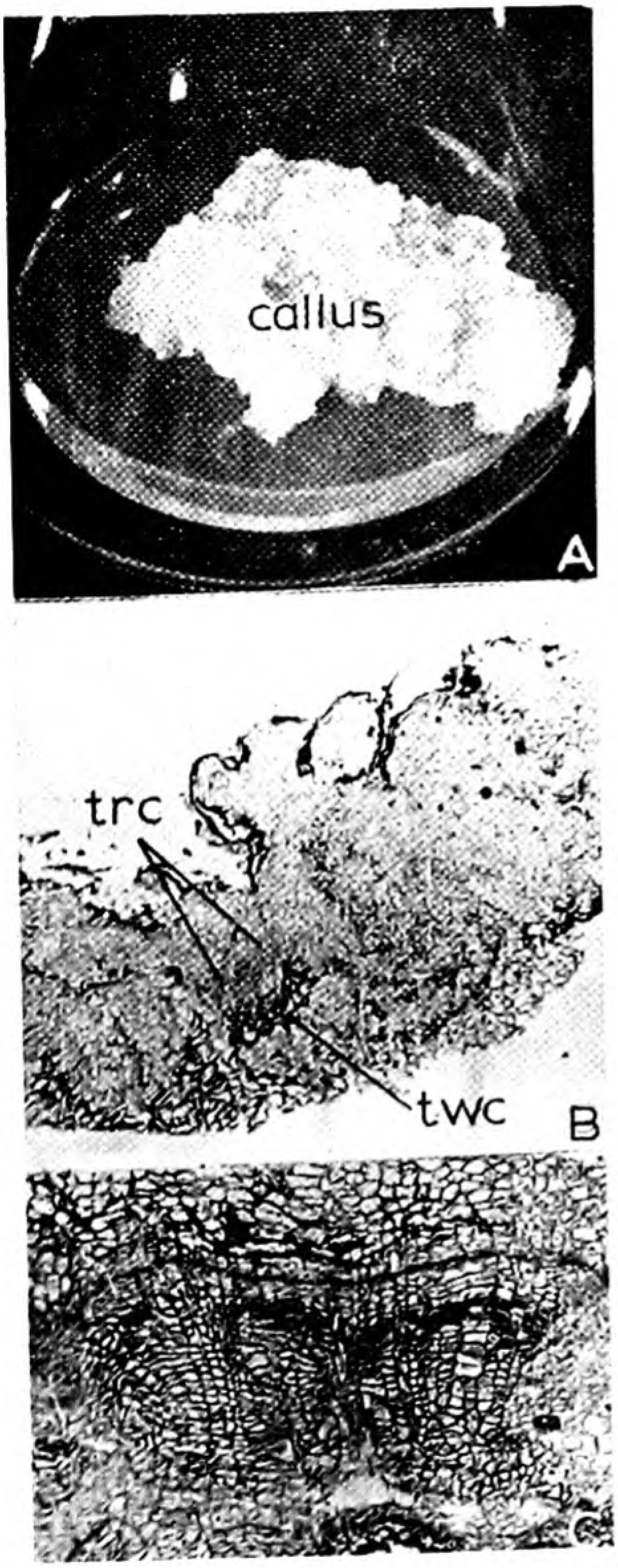


FIG. 2A–C. A. 14-week-old subcultured endosperm callus, x 2. B. Section of a 16-week-old culture to show compact callus and zonal differentiation of thick-walled cells, x 22. C. Portion of B enlarged to show the vascular bundle-like tissue, x 130.

Trc, tracheidal cells; twc, thick-walled cell.

TABLE I

INCREASE IN WEIGHT ON DIFFERENT *p*H*

Increase %	*p*H 4.5	5.0	5.5	5.8	6.0	6.5	7.0
Fresh weight	140	1320	950	750	650	610	237
Dry weight	35	350	270	190	180	140	70

* Total growth period, 6 weeks. Average values of 48 cultures.

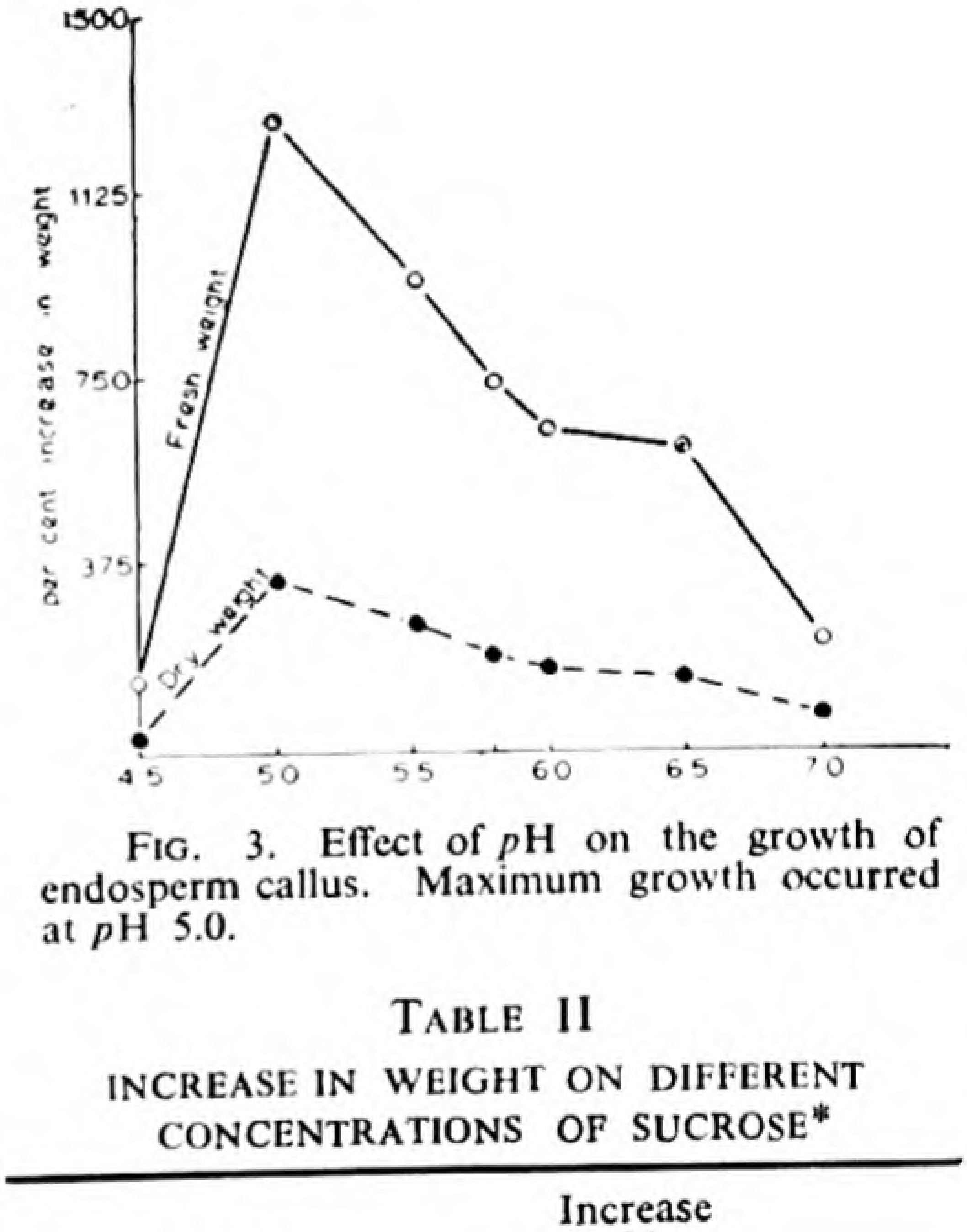


FIG. 3. Effect of *p*H on the growth of endosperm callus. Maximum growth occurred at *p*H 5.0.

TABLE II

INCREASE IN WEIGHT ON DIFFERENT CONCENTRATIONS OF SUCROSE*

Sucrose (%)	Increase % Fresh weight	Dry weight
1.0	800	215
2.0	1345	385
4.0	1440	480
6.0	1312	325
8.0	1030	285
10.0	885	235
12.0	650	185

* Total growth period: 6 weeks. Average values of 48 cultures.

A gradual increase in fresh and dry weight occurred from 1.0 to 4.0% sucrose. Further increase in sucrose concentration resulted in decrease in weight, and 12% sucrose yielded only 650% increase in fresh weight (Fig. 4).

The growth of endosperm callus was also studied on medium containing other sugars (Fig. 5; Table III).

TABLE III

INCREASE IN WEIGHT ON DIFFERENT SUGARS*

Sugar (10^{-1} M)	% Increase	
	Fresh weight	Dry weight
Arabinose	198	50
Fructose	1075	285
Galactose	198	45
Glucose	1225	320
Mannose	45	28
[illegible]	150	25
[illegible]	200	32
[illegible]	[illegible]	50
[illegible]	945	235
[illegible]	1360	375
[illegible]	[illegible]	185

* [illegible] period: 6 weeks. Average [illegible]

[illegible] that glucose, [illegible] produces better growth [illegible], 1902; Kotte, [illegible], 1937). However, White [illegible] reported that sucrose was more [illegible] than any other carbohydrate for the growth of excised tomato roots. Our experiments, as well those of many others prove that sucrose is much better for callus growth. Next to sucrose is glucose and fructose, and mannose is least effective. On arabinose, cellobiose, lactose, mannose and sorbose, at the end of 4 weeks, the tissue turned brown and collapsed.

To find out if any other phenoxyacetic acid could bring about a similar increase in growth as 2, 4-D, 3 phenoxyacetic acids were tried at 10^{-5}M. Table 4 shows the results.

TABLE IV

INCREASE IN WEIGHT ON DIFFERENT PHENOXYACETIC ACIDS*

Increase %	Phenoxyacetic acids			
	2,4,5-T	o-PAA	p-PAA	2,4-D
Fresh weight	695	765	1230	1360
Dry weight	185	192	325	430

* Total growth period: 6 weeks. Average values of 48 cultures.

While 2,4,5-T proved least effective, 2,4-D was most effective (Fig. 6). None of the phenoxyacetic acids tested, supported vascular differentiation in callus tissue. Following these results, 8 different concentrations of 2, 4-D were tested and the results are summarised in Table V.

On the basis of fresh weight, maximum growth of the callus (1415%) occurred with 2.5 ppm but, on dry weight (375%) basis with 2.0 ppm. The callus was friable with 3.0 to 5.0 ppm 2,4-D (Fig. 7). Also, the rate of cell division in the callus increased up to 4.0 ppm. On higher concentration the callus ceased to grow after 5 weeks.

Of the different concentrations of KN tested the optimal growth of the callus was obtained on WB+2,4-D (2.0 ppm) +KN (8.0 ppm)+YE (2500 ppm) (see Table VI).

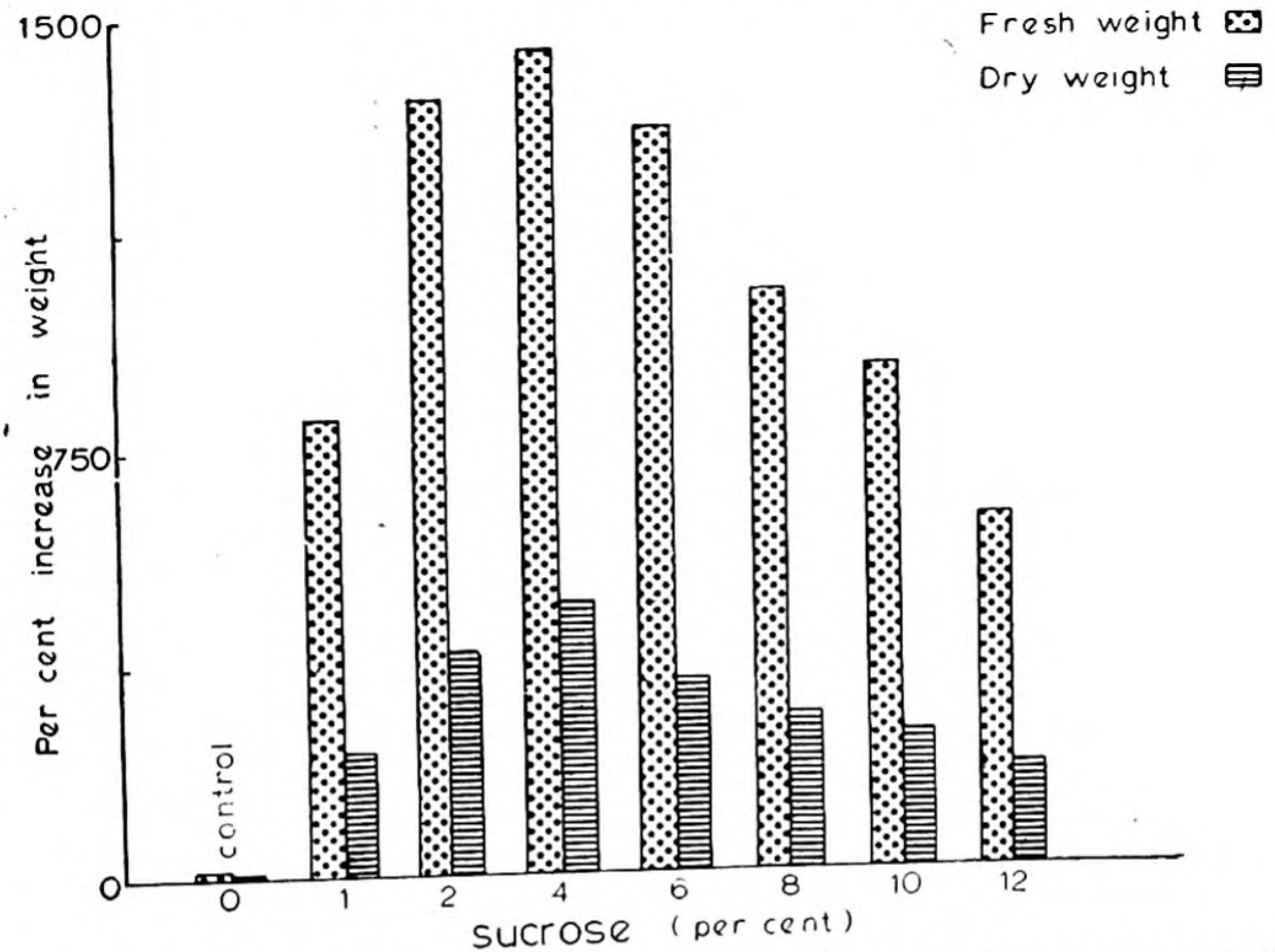


FIG. 4. Effect of sucrose on the growth of endosperm callus; 4% brought about maximum growth.

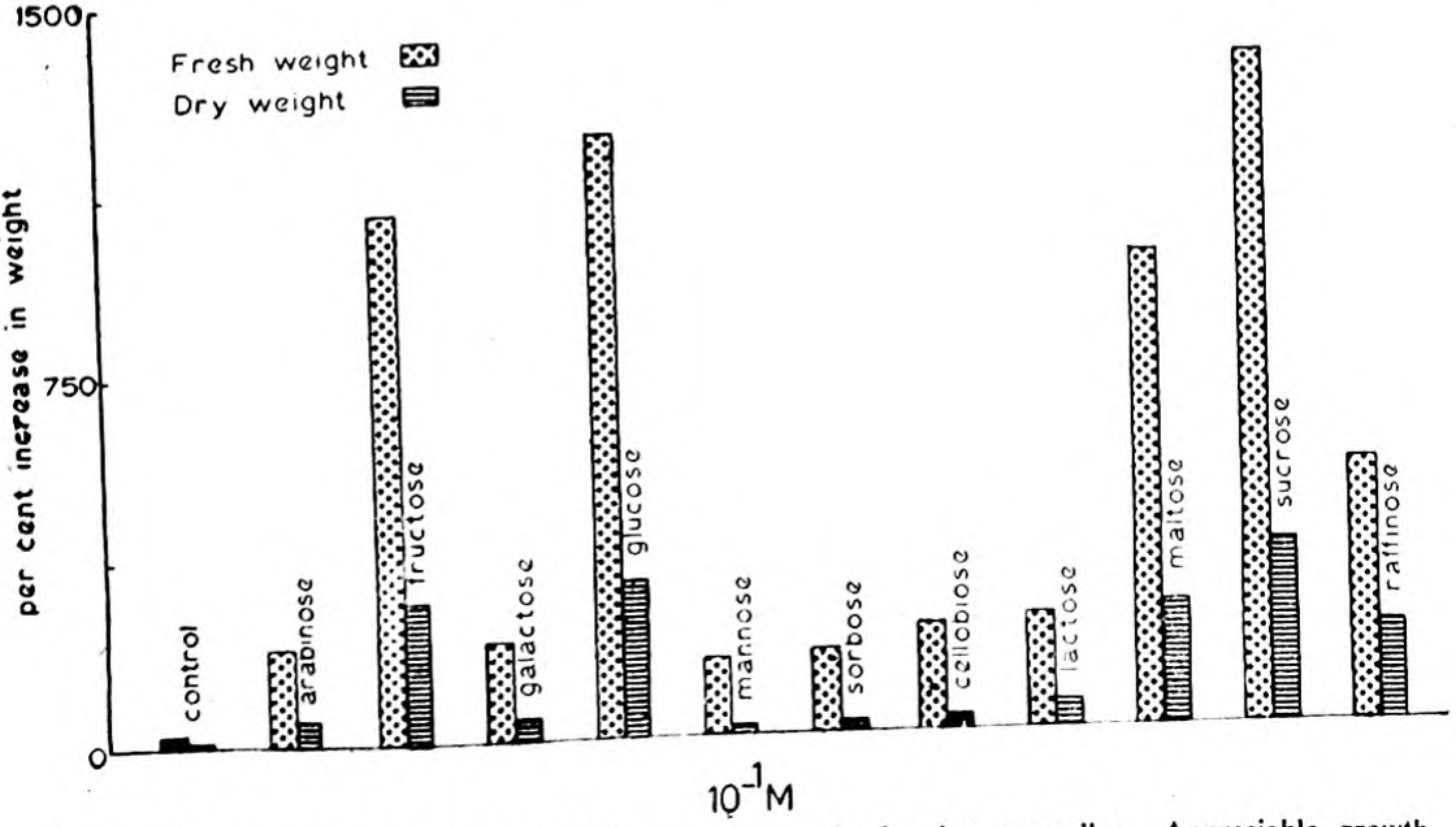


FIG. 5. Effect of different sugars (10^{-1}M) on the growth of endosperm callus. Appreciable growth occurred on sucrose, glucose, and fructose; mannose was least effective.

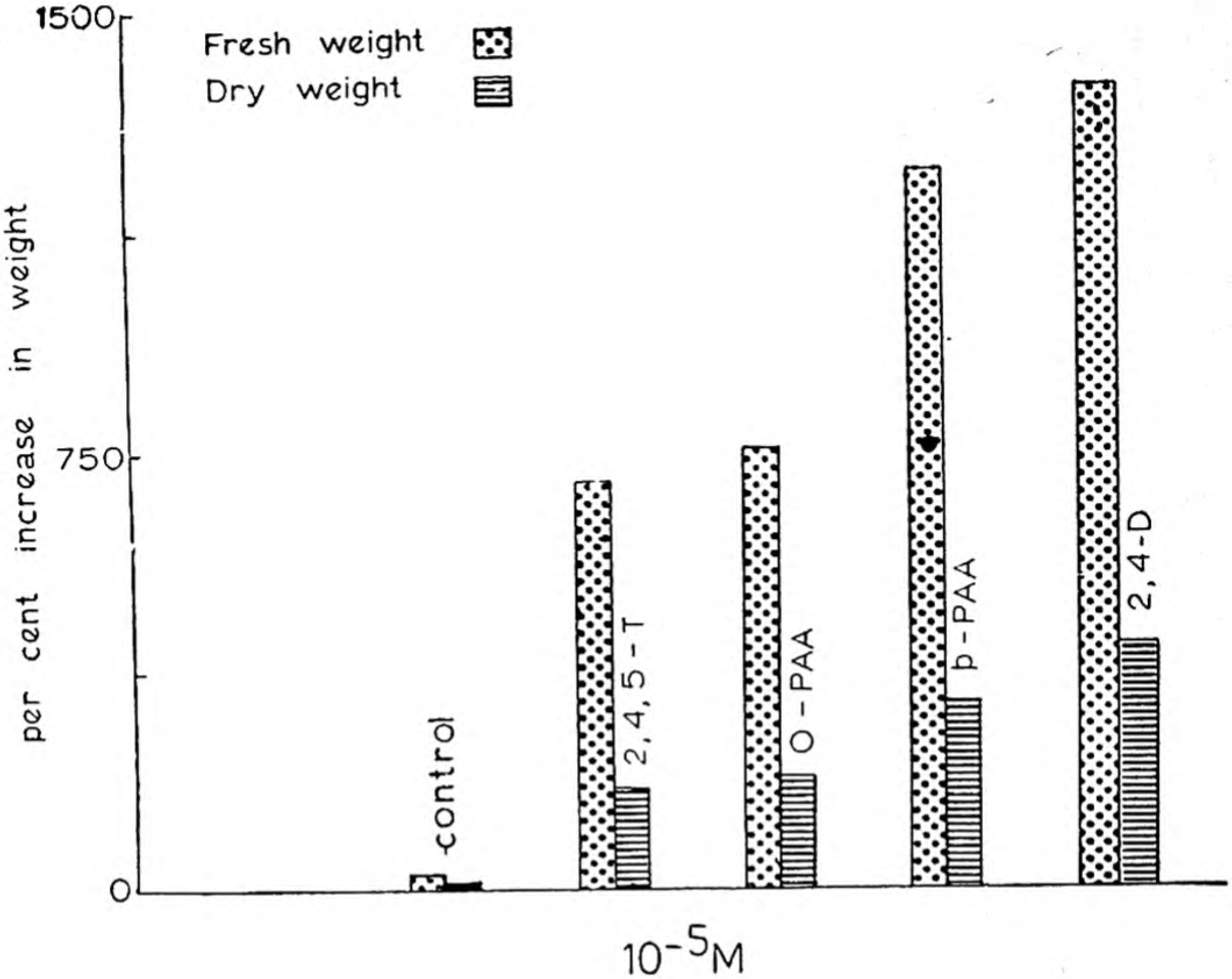


FIG. 6. Effect of some phenoxyacetic acids (10^{-5}M) on the growth of endosperm callus; 2, 4-D proved most effective and 2, 4, 5-T the least.

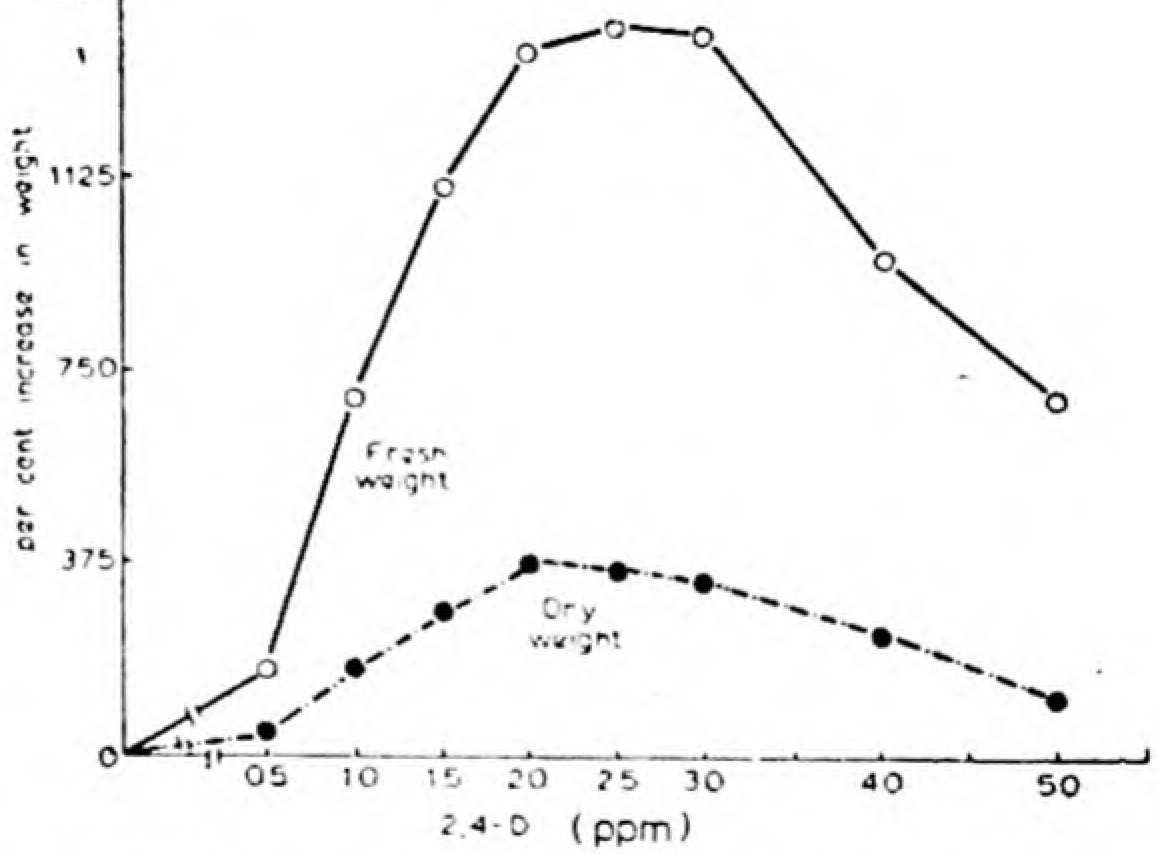


FIG. 7. Effect of 2, 4-D on the growth of endosperm callus. Maximum increase in fresh weight occurred at 2.5 ppm; on dry weight basis, at 2.0 ppm.

TABLE V

INCREASE IN GROWTH ON DIFFERENT CONCENTRATIONS OF 2, 4-D*

Increase %	2, 4-D (ppm)							
	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0
Fresh weight	180	700	1120	1360	1415	1404	955	700
Dry weight	45	140	280	375	318	325	235	100

* Total growth period: 6 weeks. Average values of 48 cultures.

Concentrations beyond 8.0 ppm proved inhibitory. If cultures were maintained under continuous light (600 lux) for 4 weeks, with 10.0 ppm KN, the callus turned slightly green.

TABLE VI

INCREASE IN WEIGHT WITH DIFFERENT CONCENTRATIONS OF KN*

KN (ppm)	Increase %	
	Fresh weight	Dry weight
1.0	316	78
2.0	788	201
5.0	1375	381
8.0	1391	373
10.0	1118	253
12.0	700	175
15.0	315	78

* Total growth period: 6 weeks. Average values of 48 cultures.

Some substituted aminopurines were also tested at 2.5×10^{-5} M. Table VII shows that maximum fresh weight increase occurred with SD 8339. KN, zeatin and 6-γ, γ also resulted in increased growth of endosperm callus; the least effective was DPU (470% fresh weight and 140% dry weight increase).

Depending upon dry weight, the increase in growth was more or less similar on DPU, triacanthine and Ad (Fig. 8).

After evaluating the optimum concentration of sugar, auxins, and substituted aminopurines, experiments were conducted to determine the optimal concentration of YE for callus growth (Fig. 9). The results are given in Table VIII. 3000 ppm YE proved most effective on fresh weight basis (1425% increase), 2500 ppm on dry weight basis (421% increase). The callus, on 3500 to 5000 ppm YE, became compact within 5 weeks; at lower concentrations (1000 to 1500 ppm) it remained friable. With 5000 ppm YE, the callus became yellow and nodulated and, finally, turned brown in 4-week-old cultures.

WB+2,4-D+KN+YE supported the growth of endosperm callus, but did not bring about any organogenesis. However, the callus could be maintained in an active state of growth even after 11 months, on WB+CH (1000 ppm). When WB+CH was supplemented with any auxin or cytokinin, the growth of the callus was appreciably slow.

An experiment was set up to compare the growth of callus on various concentrations of CH.

Minimal growth of the tissue (275% increase in fresh weight) occurred with

TABLE VII

INCREASE IN WEIGHT WITH DIFFERENT AMINOPURINES*

Increase %	Aminopurine 2.0×10^{-5} M							
	DPU	triaca-nthine	Ad	BA	6—γ, γ	zeatin	KN	SD 8339
Fresh weight	470	565	615	945	1035	1225	1335	1425
Dry weight	140	140	150	225	290	380	395	470

* Total growth period: 6 weeks. Average values of 48 cultures.

TABLE VIII

INCREASE IN WEIGHT WITH DIFFERENT CONCENTRATIONS OF YE*

Increase %	YE (ppm)								
	500	1000	1500	2000	2500	3000	3500	4000	5000
Fresh weight	335	695	875	1250	1405	1424	1125	705	280
Dry weight	95	162	227	375	421	385	290	145	56

* Total growth period: 6 weeks. Average values of 48 cultures.

5000 ppm CH (Fig. 10); the callus succumbed after 5 weeks. On both, fresh and dry weight basis, 2000 ppm proved most satisfactory.

CH is a complex mixture of amino acids and certain other substances. Two concentrations (0.7×10^{-5} and $1{\cdot}4\times10^{-5}$ M) of 12 amino acids were tested for callus growth, with an idea to replace CH. Of these amino acids, glutamic acid, valine, arginine, threonine, isoleucine, and histidine proved effective at 1.4×10^{-5} M, and lysine, leucine, tyrosine, phenylalanine, glycine and methionine at $0{\cdot}7\times10^{-5}$ M (see Table X).

TABLE IX

INCREASE IN WEIGHT WITH DIFFERENT CONCENTRATIONS OF CH*

Increase %	CH (ppm)							
	500	1000	1500	2000	2500	3000	4000	5000
Fresh weight	450	1290	1357	1404	977	710	400	275
Dry weight	133	335	370	380	275	187	90	50

* Total growth period: 6 weeks. Average values of 48 cultures.

On both, fresh (980% increase) and dry (238% increase) weight basis, glutamic acid ($1{\cdot}4\times10^{-5}$M) brought maximum growth (Fig. 11). On 0.7×10^{-5} M, only 400% increase in fresh weight occur-

TABLE X

INCREASE IN WEIGHT WITH DIFFERENT AMINO ACIDS*

Amino acids	1.4×10^{-5}M Increase % Fresh weight	 Dry weight	0.7×10^{-5}M Increase % Fresh weight	 Dry weight
Glutamic acid	980	238	410	100
Arginine	835	200	385	100
Threonine	750	155	387	80
Lysine	230	70	425	105
Leucine	175	45	410	90
Tyrosine	150	35	375	72
Isoleucine	355	70	240	65
Phenylalanine	200	50	285	62
Valine	248	60	228	65
Glycine	175	45	225	52
Histidine	155	20	100	28
Methionine	80	20	120	35

*Total growth period : 6 weeks. Average values of 48 cultures.

red. Arginine, threonine, lysine, and leucine were as good as glutamic acid at 0.7×10^{-5}M. Even a mixture of these amino acids could not bring about as much growth as CH alone.

Thus, it was possible to partly replace CH with a single amino acid.

ANATOMICAL STUDIES

Squash preparations of 4-week-old endosperm callus showed only parenchymatous cells. After another 2 weeks the tissue developed tracheidal cells. Differentiation of tracheidal cells in the endosperm callus was more pronounced with 2.0 to 4.0% sucrose, than with lower or higher concentrations. Without any sugar, there was neither any growth of the tissue nor differentiation of tracheidal

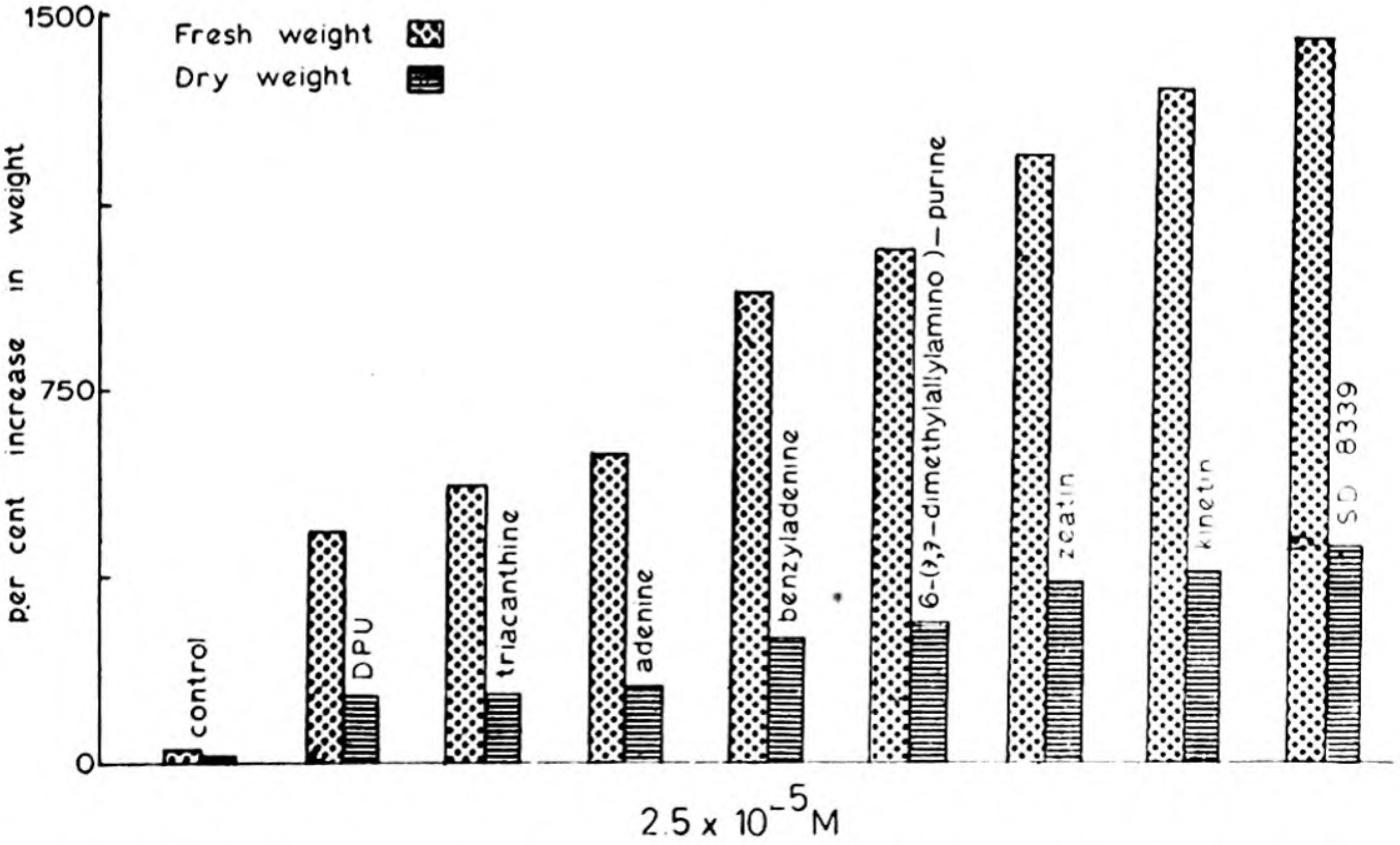


FIG. 8. Effect of some substituted aminopurines and DPU on the growth of endosperm callus. Maximum growth was obtained with SD 8339; DPU was least effective.

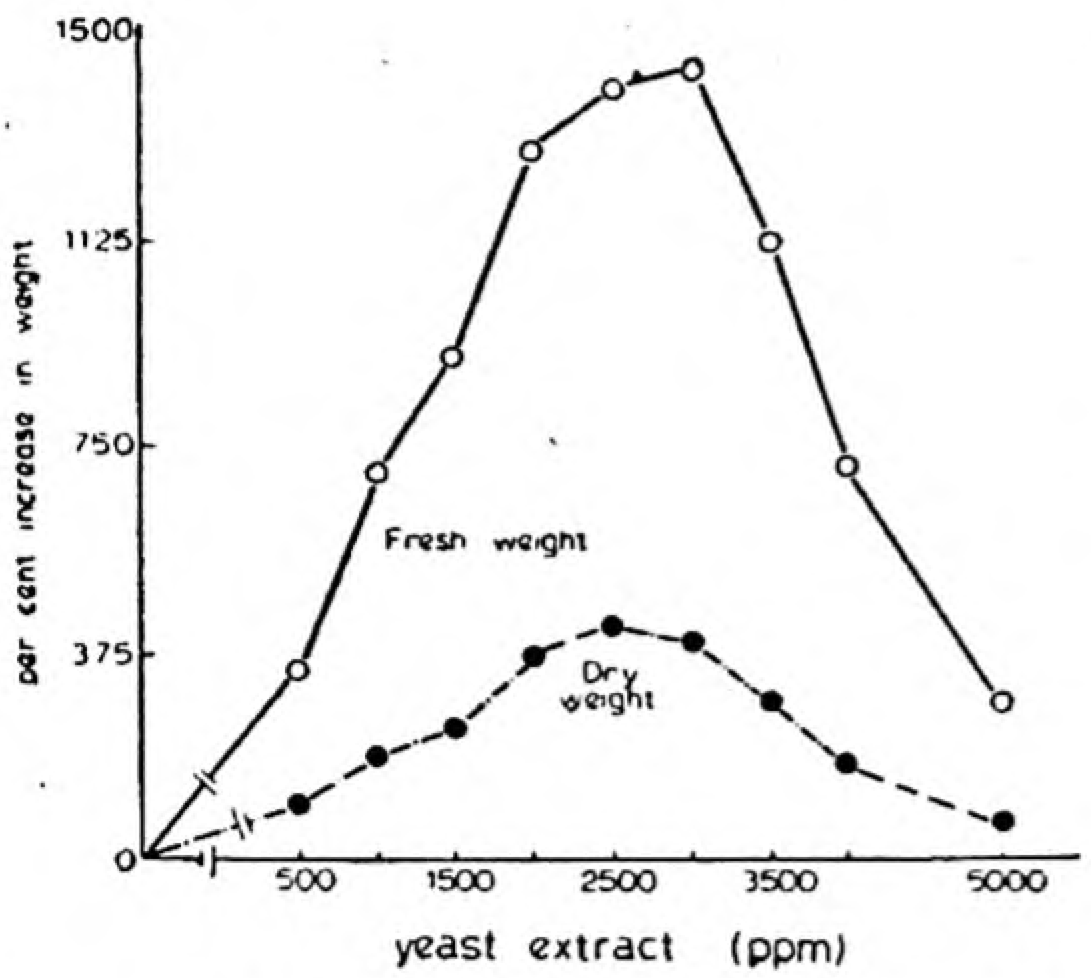


FIG. 9. Effect of YE on the growth of endosperm callus. On fresh weight basis maximum growth occurred at 3000 ppm, and on dry weight basis at 2500 ppm.

cells. A gradual decline in callus growth, and formation of tracheidal cells, was observed when the callus obtained on sucrose-medium was transferred to a sucrose-free medium. With arabinose, cellobiose, lactose, mannose and sorbose (supplemented individually in the medium), the growth of callus was poor, and tracheidal cells did not differentiate. Thickening of cell walls of the callus, and differentiation and organisation of tracheidal cells, depended on the constitution of the medium.

The disposition of thick-walled cells was, at first, irregular. Later, these were restricted to the cambium-like zones formed in the callus. In 5-month-old cultures, the organisation of tracheidal

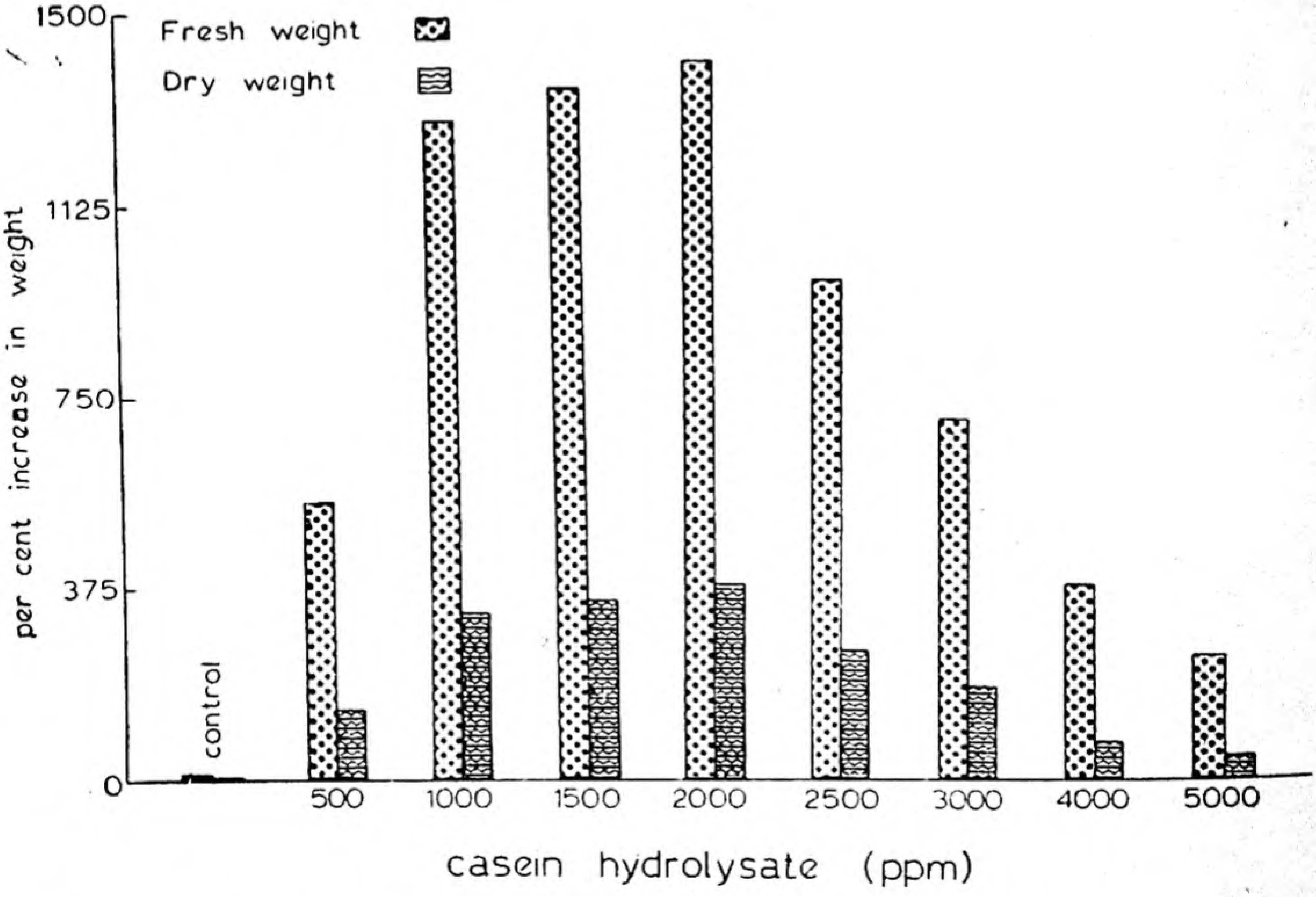


FIG. 10. Effect of CH on the growth of endosperm callus. Both on fresh and dry weight basis, maximum growth occurred at 2000 ppm, and minimum at 5000 ppm.

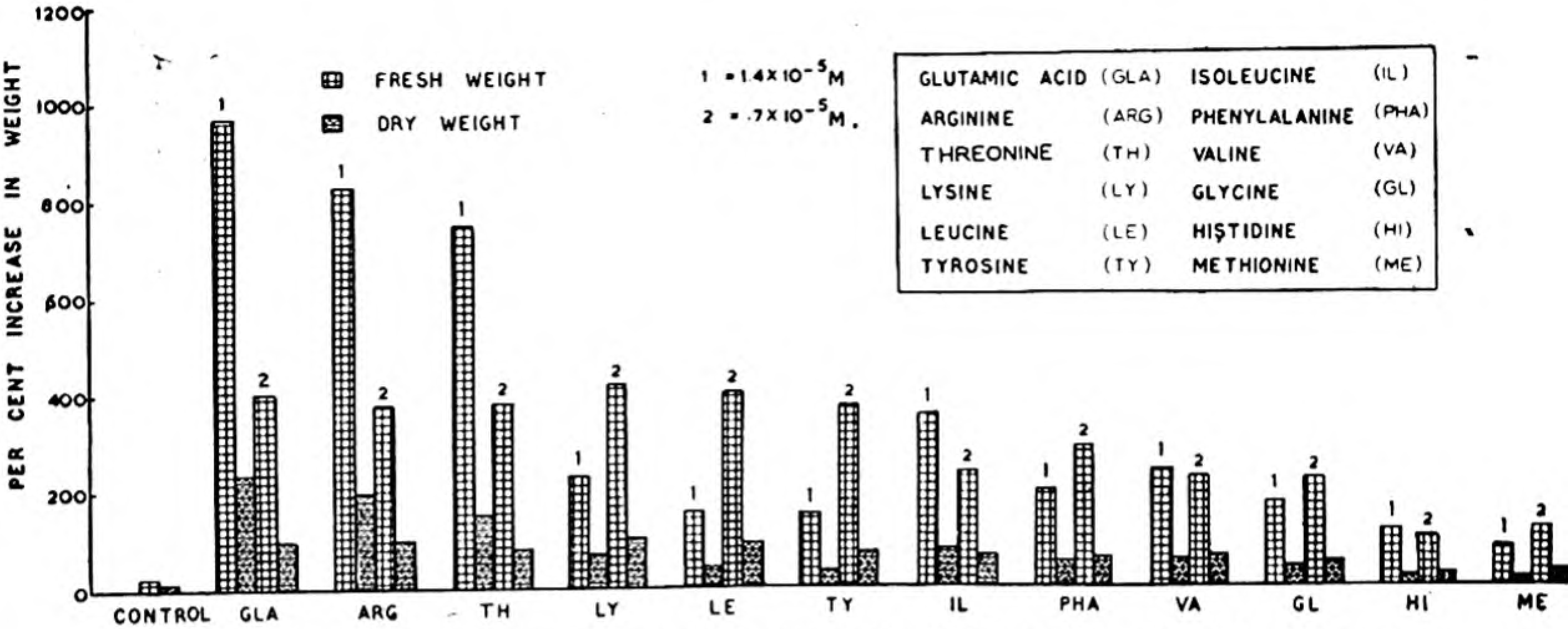


FIG. 11. Effect of some amino acids on the growth of endosperm callus. Glutamic acid was most effective at 1.4 x 10^{-5}M, and methionine the least effective.

cells and cambium layer simulated vascular bundles (Fig. 2 B, C). Phloroglucinol-HCI test, either in squash preparations or in freehand sections, revealed a positive test for lignin.

When YE was present in the medium the endosperm callus showed actively dividing cells, filament of cells, and cell clusters showing hypertrophied nuclei and fragmentation of nucleoli. Occasionally, the cell assemblages simulated stages of embroygeny in 14-week-old cultures.

The initiation of proliferation occurs by internal cell division, as well as by formation of cambium-like layers. Initially, the endosperm cells contain fat globules (Fig. 1 B, C), and there is gradual loss of reserve food during in vitro growth. Ultimately, after 10 weeks, when considerable callus has developed, there was no trace of any food reserve.

The callus also exhibited groups of vascular elements and meristematic zones. However, organogenesis failed to occur.

DISCUSSION

Very little work has been done on the growth requirements of tissue under in vitro conditions (see Eriksson, 1965; Steward and Degani, 1969). Also, the growth responses of mature endosperm have not been studied earlier. Sternheimer (1954) did not observe proliferation of waxy and starchy varieties of corn endosperm (see also Nelson and Rines, 1962). Tamaoki and Ullstrup (1958) obtained satisfactory growth of maize endosperm tissue on Nitsch's medium +YE. The proliferation of endosperm of *Cucumis* occurred on WB (5% sucrose) +YE (2000 ppm) and/or IAA (2.0 ppm) +1,3-diphenylurea (2.5 ppm)+CH (500 ppm). There was no organogenesis though groups of meristematic cells were observed frequently (see also Sehgal, 1969). A similar medium supported the growth of endosperm callus of *Ricinus communis* (present studies).

In most of the studies *p*H has not been considered as a critical factor for growth, yet, in some tissues, it has proved to be a limiting factor. The *p*H of the nutrient media is usually maintained between 4.5 to 6.3. Straus and LaRue (1954) found that, in corn endosperm cultures on a fresh weight basis, *p*H 7.0 supported best growth wheresas, on a dry weight basis, *p*H 6.1 appeared optimal. The best growth of endosperm tissue of *Ricinus* occurred on a slightly acidic medium, *p*H 5.0. Similarly, Lampton (1952) obtained 100% increase in endosperm tissue of *Asimina triloba* at *p*H 4.0, and 95% increase at *p*H 5.0. Thus, there was not much difference in growth at *p*H 4.0 or 5.0. An alkaline medium is reported to support the growth of the tissue in a very few instances (see Gautheret, 1947a, b; Sanders and Burkholder, 1948).

As a carbon source, sucrose has been commonly used in tissue cultures, and its beneficial effect has been reported in other plant tissue cultures (see McCready and Hassid, 1941; Van Overbeek et al., 1944; Burström, 1948; Leben and Keitt, 1948; Dormer and Street, 1949; Street and Lowe, 1950; Hildebrandt and Riker, 1953; Boll, 1954; Beevers, 1961; Loewus et al., 1962). The decrease in growth with higher concentration of sucrose can be explained by the experiments of Street and Lowe (1950) who found that the effect of 4% sucrose in the inhibition of growth was not due to the osmotic pressure of the medium. Glucose and fructose have also proved suitable for callus growth (see Homes, 1967), but their efficiency for supporting the growth of endosperm, varied considerably.

Glucose proved to be the best carbon source for rice callus tissue, and was slightly better than fructose, sucrose and maltose (Yatazawa and Furuhashi, 1968). Nickell and Maretzki (1970) obtained best growth of the stem tissue of sugarcane with sucrose, glucose, fructose, cellobiose, melibiose, trihalose, raffinose, and ribose, a pentose sugar, which is not generally utilized by plant tissues in cultures (see also Gautheret, 1955). *Ricinus* endosperm was also unable to utilize galactose, lactose, arabinose, and raffinose. Our findings support the report of Straus and LaRue (1954) on corn endosperm cultures.

Tomato juice promoted the growth of endosperm of corn (LaRue, 1949; Sternheimer 1954), but the growth was erratic and unpredictable because the kinin-like activity of tomato juice changes correspondingly with the age of the fruit (Bottomley et al., 1963), The addition of complex substances like TJ, CM, CH, YE and extract of many seeds add to the activity of the medium (Pieczur, 1952; Jablonski and Skoog, 1954; Matsubara, 1962; Kuo and Wang, 1964; Sussex and Frei, 1968), but the nature of stimulatory substances contained therein has not yet been entirely elucidated. Straus and LaRue (1954) and Tamaoki and Ullstrup (1958) observed that YE could fairly replace TJ for the growth of corn endosperm callus. YE is now known to be essential for the satisfactory growth of mature endosperm. This is also true of *Croton* (Bhojwani, 1966), *Cucumis* (Nakajima, 1962), *Lolium* (Norstog, 1956), *Santalum* (Rangaswamy and Rao, 1963), and *Ricinus* (present studies). CH is not comparable to YE for callus growth. The requirement of high concentration of YE by *Ricinus* endosperm is in contrast with many tissues studied by others (See Robbins, 1922; White, 1932, 1934, 1937a, b; Robbins and White, 1936; Wetmore and Wardlaw, 1951; Lampton, 1952; Steeves and Sussex 1952).

The mature endosperm tissue cultures, thus far established, show optimal growth on a medium containing an auxin, a cytokinin, and YE or CH.

An attempt was made to replace CH with a single amino acid. Straus and LaRue

(1954) and Straus (1960) found that addition of 1.5×10^{-2}M asparagine to WM resulted in better growth of maize endosperm. Arginine was ineffective; glutamine, glutamic acid and aspartic acid (1.5×10^{-2}M) supported appreciable growth. However, in our experiments on the growth of endosperm callus, glutamic acid proved most satisfactory. The fact that glutamic acid is utilized by the growing endosperm callus is further strengthened by the findings of Stewart and Beevers (1967); of the 4 amino acids compared, aspartate, glutamate and alanine proved better sugar precursors than valine.

In corn endosperm tissue Straus (1960) reported that aspartic acid and cysteine promoted pigment synthesis, while riboflavin, methionine, asparagine, glutamine and valine inhibited this. Aspartic acid played a significant role in anthocyanin synthesis. In the present studies, however, in spite of the fact that all the above mentioned amino acids were used, pigment formation did not occur.

Straus (1959), working with corn endosperm tissue, suggested that, in general, acid media have small promotive effect on pigment synthesis but alkaline media definitely have an inhibitory effect. The endosperm callus of *Ricinus*, grown on a slightly acidic medium (*p*H 4.5 -5.0), did not show any pigmentation. Sucrose is also reported to permit greater pigment synthesis (Straus, 1959) than either glucose or fructose. Higher concentrations of sucrose do not result in increased pigment production. No such correlation was observed by us in the endosperm tissue cultured on high sucrose medium.

In endosperm, as well as in many other tissues, the initiation of proliferation takes place by laying down of a 'cambium' layer, or by internal divisions (see Chen and Galston, 1967; Noel, 1968; Robards et al., 1969). The cell division begins only after a lag phase, and the initial divisions are more prominent in the peripheral cells. In *Osyris* (Johri and Bhojwani, 1965), localized peripheral meristems lead to the formation of surface outgrowths in which large patches of tracheidal cells differentiate. This is also true of the endosperm callus of *Ricinus communis* (present studies).

The formation of tracheidal cells in cultured endosperm tissue was comparable to many other tissues. Rier and Beslow (1967) mention a correlation in the quantity of xylem and sucrose concentration; a similar correlative effect was observed in the present studies on the endosperm callus of *Ricinus*. Arcs of tracheidal cells were present in *Ricinus* at 2.0% sucrose, the areas of these xylary elements were greater at higher concentration. Each arc was bordered by an internal cambium; this is also true of other tissues (Rier and Beslow, 1967; Beslow and Rier, 1969). Siegal (1962) also proposed that the influence of sucrose upon the induction of tracheids in callus is inter-related.

The importance of carbon sources and auxins for the differentiation of vascular tissues have been reported by Torrey (1953), Clutter (1960), Rier (1962), Wetmore et al. (1964) and Jeffs and Northcote (1967) (see also Roberts, 1969).

Effect of 2, 4-D on tracheid differentiation has been studied by Fosket and Roberts (1964) in *Coleus*. Ganapathy and Hildebrandt (1967) observed increased lignification with the addition of 2,4-D. In *Phaseolus* and *Coleus* also, 2,4-D and 2,4,5-T stimulated xylogenesis (Jacobs, 1967). It is also known to stimulate shoot formation and rooting in many plants (Muzik and Cruzado, 1958; Reinert, 1959; Nakajima and Yamaguchi, 1967; Rao and Narayanawami, 1968; Nataraja, 1968; Matsushima et al., 1969; Petrů, 1970).

2, 4-D, however, did not bring about any significant change in the lignification and organogenesis of endosperm callus.

Thus, the cells of the mature endosperm of autotrophic angiosperms can be induced to proliferate in cultures under the influence of suitable chemical milieu and the tissue thus derived possesses the potentiality of unlimited growth in vitro. The essentiality of CH can in part be replaced by a single amino acid.

References

Beevers, H. 1961. Metabolic production of sucrose from fat. *Nature, Lond.* **191** : 433-436.

Beslow, D. T., and J. P. Rier, 1959. Sucrose concentration and xylem regeneration in *Coleus* internodes in vitro. *Pl. Cell Physiol., Tokyo* **10:** 69-76.

Bhojwani, S. S. 1966. Morphogenetic behaviour of mature endosperm of *Croton bonplandianum* in culture. *Phytomorphology* **16:** 349-353.

——. 1968. *Morphogenetic Studies on Cultured Mature Endosperm and Embryo of some Angiosperms.* Ph. D. Thesis, Univ. Delhi.

Boll, W. G. 1954. Effect of sucrose concentration, length of passage, minor element nutrition and *p*H value on growth of excised tomato roots. *Bot. Gaz.* **116:** 156-162.

Bottomley, W., N. P. Kefford, J. A. Zwar, and P. L. Goldacre. 1963. Kinin activity from plant extracts. *Aust. J. boil. Sci.* **16:** 395-406.

Brink, R. A., and D. C. Cooper. 1947. The endosperm in seed development. *Bot. Rev.* **13:** 423-541.

Burström, H. 1948. Observations on the influence of galactose on wheat roots. *Physiologia Pl.* **1:** 209-215.

Chen, H., and A. W. Galston. 1967. Growth and development of *Pelargonium* pith cells in vitro. II., Initiation of organized development. *Physiologia Pl.* **20**: 533-539.

Clutter, M. E. 1960. Hormonal induction of vascular tissues in tobacco pith in vitro. *Science, Wash.* **132:** 548-549.

Dormer, K. J., and H. E. Street. 1949. The carbohydrate nutrition of tomato roots. *Ann. Bot.* **13:** 199-217.

Eriksson, T. 1965. Studies on the growth requirements and growth measurements of cell cultures of *Haplopappus gracilis. Physiologia Pl.* **18:** 976-993.

Fosket, D. E., and L. W. Roberts. 1964. Induction of wound vessel differentiation in isolated *Coleus* stem segments in vitro. *Am. J. Bot.* **51:** 19-25.

Ganapathy, P. S., and A. C. Hildebrandt. 1967. Suspension cultures and lignification in *Lactuca sativa L. Indian J. Expl. Biol.* **5:** 196-197.

Gautheret, R. J. 1947a. *p*H et culture de tissus végétant. *Revue. gèn. Bot.* **54:** 5-34.

——. 1947b. Action du *p*H sue le développement des cultures de tissus de carotte. *C. r. Soc. Biol., Paris.* **141:** 25.

——. 1955. The nutrition of plant tissue cultures. *A. Rev. Pl. Physiol.* **6:** 433-484.

Haberlandt, G. 1902. Culturveruche mit isolierten pflanzenellen. Sitz-Ber. *Mat.-Nat. Kl. Kais. Alad. Wiss., Wien* **111:** 69-92.

Hildebrandt, A. C., and A. J. Riker. 1953. Influence of concentrations of sugars and polysaccharides on callus tissue growth in vitro. *Am. J. Bot.* **40:** 66-76.

Homes, J. 1967. Action morphogenetique du glucose sur une souche embryogene de tissus de carotte cultivee in vitro. *C. r. Seanc. Soc. Boil. Filliales* **161** : 1143-1145.

Jablonski, J. R., and F. Skoog. 1954. Cell enlargement and cell division in excised tobacco pith tissue. *Physiologia Pl.* **7:** 16-24.

Jacobs, W. P. 1967. Comparison of the movement and vascular differentiation effects of the endogenous auxin and of phenoxyacetic weedkillers in stems and petioles of *Coleus* and *Phaseolus. Ann. N. Y. Acad. Sci.* **144:** 102-117

Jensen, W. A. 1962. *Botanical Histochemistry.* W. H. Freeman and Co., San Francisco.

Jeffs, R. A., and D. H. Northcote. 1967. The influence of IAA and sugar on the pattern of induced differentiation in plant tissue cultures. *J. Cell. Sci.* **2**: 77-88.

Johri, B. M., and S. S. Bhojwani. 1965. Growth responses of mature endosperm in cultures. *Nature, Lond.* **208** : 1345-1347.

Kotte, W. 1922. Wurzelmeristem in gewebekulture. *Ber. Deut. Bot. Ges.* **40:** 269-172.

Kuo, J. S., and F. H. Wang. 1964. The effect of coconut milk, casein hydrolysate and glutamine on the growth of the sunflower young embryos grown in vitro. *Acta. bot. sinica.* **12:** 364-372.

Krikorian, A. D., and D. L. Berquam. 1969. Plant cell and tissue cultures. The role of Haberlandt. *Bot. Rev.* **35:** 59-67.

Lampton, R. K. 1952. *Developmental and Experimental Morphology of the Ovule and Seed of Asimina triloba.* Ph. D. Thesis, Univ. Michigan, Ann Arbor, USA.

La Rue, C. D. 1949. Culture of endosperm of maize. *Am. J. Bot.* **36:** 798 (Abstr.).

Leben, C., and G. W. Keitt. 1948. *Venturia inaequalis* (cke). Wint. V. The influence of carbon and nitrogen sources and vitamins on growth in vitro. *Am. J. Bot.* **35:** 337.

Loewus, F. A., S. Kelly, and E. F. Neufeld. 1962. Metabolism of myo-inositol in plants: Conversion to pectin, hemicellulose, D-xylose and Sugar acids. *Proc. natn. Acad. Sci.,* **USA 48:** 421-425.

MAHESHWARI, P. 1950. *An Introduction to the Embrology of Angiosperms.* McGraw Hill Book Co., Inc., New York.

MATSUBARA, S. 1962. Studies on a growth-promoting substance "Embryo factor" necessary for the culture of young embryos of *Datura tubula* in vitro. *Bot. Mag., Tokyo* **75:** 10-18.

MATSUSHIMA, H., M. WADA, AND M. TAKEUCHI. 1969. The crystal containing body in tobacco cultured cells. *Bot. Mag., Tokyo* **82:** 417-423.

MCCREADY, R. M., AND W. E. HASSID. 1941. Transformation of sugars in excised barely shoots. *Pl.Physiol., Lancaster* **16:** 599-610.

MUZIK, T. J., AND H. J. CRUZADO. 1958. Differentiation of bean internode segments in tissue cultures with added indoleacetic acid, 2,4- dichlorophenoxyacetic acid, and maleic hydrazide. *Bot. Gaz.* **120:** 57-59.

NAG, K. K. 1970. *Morphogenic Studies on Endosperm, Embryo, and other Sporophytic Tissues of some Parasitic Angiosperms.* Ph. D. Thesis, Univ. Delhi.

NAKAJIMA, T. 1962. Physiological studies of seed development, especially embryonic growth and endosperm development. *Bull. Univ. Osaka Prefect. Ser.* B. **13:** 13-48.

——, AND T. YAMAGUCHI. 1967. On the embryogenesis, observed in tissue culture of carrot, *Daucus carota.* L. *Bull. Univ. Osaka Prefect. Ser.* B **19:** 43-49.

NATARAJA, K. 1968. *Morphogenetic Studies In Vitro on Some Members of the Ranunculaceae.* Ph. D. Thesis, Univ. Delhi.

NELSON, O. E. AND H. W. RINES. 1962. The enzymatic deficiency in the waxy mutant of maize. *Biochem. Biophysic. Res. Comm.* **9:** 297-300.

NICKELL, L. G., AND A. MARETZKI. 1970. The utilization of sugars and starch as carbon sources by sugarcane cell suspension cultures. *Pl. Cell. Physiol.* **11:** 183-185.

NOEL, A. R. A. 1968. Callus formation and differentiation at an exposed cambial surface. *Ann. Bot.* **32:** 347-359.

NORSTOG, K. J. 1956. Growth of rye- Grass endosperm in vitro. *Bot. Gaz.* **117:** 253-259.

PETRŮ, E. 1970. Development of embryoids in carrot root callus culture (*Daucus carota* L.) *Biologia Pl.* **12** : 1-5.

PIECZUR, E. A. 1952. Effect of tissue cultures of maize endosperm on the growth of excised maize embryos. *Nature, Lond.* **170:** 241.

RAGHAVAN, V. 1966. Nutrition, growth and morphogenesis of plant embryos. *Biol. Rev.* **41**: 1-58.

RANGASWAMY, N. S., AND P. S. RAO. 1963. Experimental studies on *Santalum album.* Establishment of tissue culture of endosperm. *Phytomorphology* **13:** 450-454.

RAO, P. S., AND S. NARAYANASWAMY. 1968. Induced morphogenesis in tissue cultures of *Solanum xanthocarpum. Planta, Berl.* **81:** 372-375.

REINERT, J. 1959. Über de kontrolle der morphogenese und die induction von adventiveembryonen an gewebekulturen aus karotten. *Planta, Berl.* **53:** 318-333.

RIER, J. P. 1962. The differentiation of vascular tissues in callus of angiosperms. *Am. J. Bot.* **49:** 663.

——, AND D. T. BESLOW. 1967. Sucrose concentration and the differentiation of xylem in callus (*Parthenocissus tricuspidata*). *Bot. Gaz.* **128:** 73-77.

ROBARDS, A. W., ELAINE DAVIDSON, AND PARVENE KIDWAI. 1969. Short-term effects of some chemicals on cambial activity. *J. expl. Bot.* **20:** 912-920.

ROBBINS, W. J. 1922. Cultivation of excised root tips and stem tips under sterile conditions. *Bot. Gaz.* **65:** 543-551 .

——, AND M. A. BARTLEY. 1937. Use of dextrose by excised tomato roots. *Science, Wash.* **86:** 290-291.

——, AND V. B. WHITE. 1936. Limited growth and abnormalities in excised corn root tips. *Bot. Gaz.* **98:** 209-242.

ROBERTS, L. W. 1969. The initiation of xylem differentiation. *Bot. Rev.* **35:** 201-250.

SANDERS, M. E., AND P. R. BURKHOLDERS. 1948. Influence of amino acids on growth of *Datura* embryos in culture. *Proc. natn. Acad. Sci., USA* **34:** 516-526.

SARGANT, E. 1900. Recent work on the results of fertilization on angiosperms. *Ann. Bot.* **14:** 689-712.

SEHGAL, C. B. 1969. Experimental studies on maize endosperm. *Beitr. Biol. Pfl.* l. **46:** 233-238.

SIEGEL, S. M. 1962. *The Plant Cell Wall.* Pergamon Press, New Yrok.

STEEVES, T. A., AND I. M. SUSSEX. 1952. In vitro culture of a fern callus. *Nature, Lond.* **170:** 672.

STERNHEIMER, P. ELIZABETH 1954. Method of culture and growth of maize endosperm in vitro. *Bull. Torrey. bot. Club* **81:** 111-113.

STEWARD, F. C., AND N. DEGANI. 1969. Endogenous characteristics of different clones of carrot explants and their exogenous requirements for growth. *Ann. Bot. N. S.* **33:** 615-646.

STEWART, C. R., AND H. BEEVERS. 1967. Gluconeogenesis from amino acids in germinating castor bean endosperm and its role in transport to the embryo. *Pl. Physiol., Lancaster* **42:** 1587-1595.

STRAUS, J. 1959. Anthocyanin synthesis in corn endosperm tissues cultures. I. Identity of the pigments and general factors. *Pl. Physiol., Lancaster* **34:** 536-541.

—. 1960a. Anthocyanin synthesis in corn endosperm. II. Effect of certain inhibitory and stimulatory agents. *Pl. Physiol., Lancaster* **35:** 645-650.

——. 1960b. Maize endosperm tissue grown in vitro. III. Development of a synthetic medium. *Am. J. Bot.* **47:** 641-647.

——., AND C. D. LARUE. 1954. Maize endosperm tissue grown in vitro. I. Culture requirements. *Am. J. Bot.* **41:** 687-694.

STREET, H. E., AND J. S. LOWE. 1950. The carbohydrate nutrition of tomato roots. II. The mechanism of sucrose absorption by excised roots. *Ann. Bot. N. S.* **16:** 185-205.

SUSSEX, I. M., AND K. A. FREI. 1968. Embryoid development in long term tissue cultures of carrot. *Phytomorphology* **18:** 339-349.

TABATA, M., AND F. MOTOYOSHI. 1965. Hereditary controls of callus formation in maize endosperm cultured in vitro. *Jap. J. Genet.* **40:** 343-355.

TAMAOKI, T., AND A. J. ULLSTRUP. 1958. Cultivation in vitro of excised endosperm and meristem tissues of corn. *Bull. Torrey bot. Club* **85:** 260-272.

TORREY, J. G. 1953. The effect of certain metabolic inhibitors on vascular tissue differentiation in isolated pea roots. *Am. J. Bot.* **40:** 525-533.

VAN OVERBEEK, J., M. E. CONKLIN, AND A. F. BLAKESLEE. 1942. Cultivation in vitro of small *Datura* embryo. *Am. J. Bot.* **29:** 472-477.

——, R. SIU, AND A. J. HAAGENSMIT. 1944. Factors affecting the growth of *Datura* embryos in vitro. *Am. J. Bot.* **31:** 219-224.

WETMORE, R. H., AND C. W. WARDLAW. 1951. Experimental morphogenesis in vascular plants. *A. Rev. Pl. Physiol.* **2:** 269-292.

——, A. E. DEMAGGIO, AND J. P. RIER. 1964. Contemporary outlook on the differentiation of vascular tissues. *Phytomorphology* **14:** 203--217.

WHITE, P. R. 1932. Influence of some environmental conditions on the growth of excised root tips of wheat seedling in liquid media. *Pl. Physiol., Lancaster* **7:** 613-628.

——. 1937a. Survival of isolated tomato roots at suboptimal and supraoptimal temperature. *Pl. Physiol., Lancaster* **12:** 771-776.

——. 1937b. Separation from yeast extract of materials essential for growth of excised tomato roots. *Pl. Physiol., Lancaster* **12:** 777-791.

——. 1943. Potentiality of unlimited growth of excised tomato root tips in a liquid medium. *Pl. Physiol., Lancaster* **9:** 586-600.

——. 1963. *A Handbook of Plant Tissue Culture*. Jacques Cattell Press, Lancaster, Pa, USA.

YATAZAWA, M., AND K. FURUHASHI. 1968. Nitrogen sources for the growth of rice callus tissue. *Soil. Sci. Pl. Nutrition* **14:** 73-79.

Adv. Pl. Morph. 1972: 359-369.

THE ROLE OF ONTOGENETIC EVIDENCE IN FLORAL MORPHOLOGY

BY SHIRLEY C. TUCKER

Botany Dept., College of Arts and Sciences, Louisiana State University, Baton Rouge, Louisiana–70803, U.S.A.

ABSTRACT

Information has been assembled to suggest that there is a relationship between centrifugal stamen initiation and the following features: fasciculate stamens, dendritically branching stamen-trunk bundles (either amphicribral, protostelic, or siphonostelic pattern). Of 32 known families containing some taxa with centrifugal stamen initiation, 22 also are reported to have some members with stamen trunk bundles, and 14 are known to have stamen fascicles in some taxa. Data for anomalous trunk-bundle structure are too limited to be meaningful. Our second point is that there is probably a developmental basis for the associated characteristics, since the assemblage occurs in both dicotyledons and monocotyledons. The limited volume of meristem within which new stamen primordia and their traces can differentiate is surrounded by maturing tissues, thereby severely limiting the possible sources of procambial traces at the time the stamen primordia are initiated on the meristem.

Centrifugal stamens occur most commonly in members of Dilleniales, Malvales, Rhoeadales, Chenopodiales, Geraniales, Parietales, Theales, Opuntiales, and some Myrtales, and in the Butomales of the Monocotyledonae. The monocotyledonous representation suggests that the centrifugal androecium has occurred more than once in phylogeny of angiosperms. More study is needed of obdiplostemonous examples, as some of these do not show clearcut centrifugal initiation when studied in detail. They tend not to show the associated characteristics and may have little in common with polystemonous members showing centrifugality.

Examples are discussed in which ontogenetic evidence has proved useful to resolve morphological problems. Complex vasculature is best approached developmentally, because young stages are relatively simple. Questions concerning ovule position, and complex vasculature of receptacle and fruit are best approached through ontogenetic studies.

The ontogenetic approach has recently attracted new interest in floral morphological problems. It provides a way of assessing structural anomalies such as centrifugal stamens, residual vascular tissue, complex or atypical vasculature, and ovule position and vascular supply. The purpose of this paper is to discuss ontogenetic studies of significance and to show their usefulness to floral morphology.

As the classical theory compares the flower to a vegetative shoot, one can also compare their developmental patterns. They share such characteristics as acropetal initiation of lateral appendages, a terminal apical meristem which produces the appendages, phyllotaxic order, and plastochronic (or periodic) production of parts. Flowers differ from vegetative shoots in their determinate habit with ultimate loss of meristematic activity, and in the reproductive function; the latter alters the patterns of development of every component of the flower. A particular value of ontogenetic studies is that young stages of flowers and floral parts are invariably simpler, with simpler vasculature, than the mature counterparts. There also are significant temporal intervals, for example, between the initiation of vascular bundles in an organ, which can be evaluated as evidence but are not detectable in mature flowers. The purpose of this paper is to discuss several current problems in which ontogenetic evidence is significant.

Centrifugal androecium.—In members of 32 plant families, the order of stamen initiation differs radically from the centripetal (acropetal) direction which botanists consider the usual pattern. In these exceptional flowers, floral parts arise acropetally except that a circular cushion of meristem remains between the first-produced stamens and the petals to the outside. After all other floral appendages form, additional stamens form on this cylindrical meristem, in a centrifugal (basipetal) direction. Payer (1857) illustrated examples of centrifugal stamen initiation in about 15 families, and Hirmer (1918) described it in the androecium of members of five families. The significance of centrifugality was however overlooked until Corner (1946) showed that there was a correlation with fascicled arrangement of the mature stamens. As evidence from development accumulates, it appears that several unusual features are associated in plants with a centrifugal androecium: fascicled arrangement of mature stamens, dendritic trunk vascular bundles supplying the stamens, and concentric organization within these trunk bundles. Table 1 summarizes the families showing some of these characteristics, and also shows gaps in our knowledge. Centrifugal stamen initiation occurs in 32 families, although some include obdiplostemonous members in which only one whorl of stamens initiates after the first whorl. Investigators report some doubt about the relationship between the two whorls, and these groups may upon investigation appear out of place among other centrifugal groups. Although information on all categories is not available for every family, 14 have stamen fascicles and 22 have stamen-trunk vascular bundles. The occurrence of the assemblage of characteristics in two Butomaceous taxa (Kaul, 1967, 1968) is particularly remarkable, as previously the features around centrifugality have been considered strictly dicotyledonous. Now that they also are known for monocotyledons, the problem is moot as to whether centrifugal initiation has arisen more than once. Evidence for centrifugality in Commelinaceae is somewhat conflicting; Payer (1857) showed centrifugal development of the two stamen-whorls in *Tradescantia*, but Rohweder (1963) was unable to confirm this pattern in *Gibasis* and several other genera of the family. He found that members of the two stamen whorls are initiated very closely in time and in level.

Both centrifugal and centripetal patterns of stamen initiation are reported in Dilleniaceae, for different genera (Corner, 1946). *Geranium* is reported (Eames, 1961) to include both types of development, in different species. Ontogenetic comparisons of such closely related taxa would perhaps elucidate how these two patterns first become distinguishable.

The Ranalian genus *Drimys* was erroneously cited as having centrifugal stamen initiation (Eames, 1961), on the basis of the innermost stamens being largest and dehiscing first. Neither condition is dependable to show the direction of initiation. In *Drimys*, the stamens are initiated centripetally (Tucker, 1959; Tucker and Gifford, 1966a).

Several taxa in Table 1 have obdiplostemonous arrangement, in which an inner antesepalous whorl of stamens is initiated before the outer antepetalous whorl. This condition is often considered reduced phylogenetically, particularly as it represents a departure from the usual alternating whorled condition. Various explanations have been offered for obdiplostemony (Eames, 1961; Puri, 1951), but here it is mainly of interest as one of several characteristics associated with centrifugality. Order of stamen initiation in obdiplostemonous flowers is often ambiguous or difficult to determine, as Leins (1964c) illustrates in his comparative study of development in several Ericalean plants. Leins suggests that the longer the temporal gap between initiation of stamen whorls, the more evident that they are discrete whorls, rather than a single whorl. In Leins' work, ontogenetic studies showed that there is no true obdiplostemony in the Ericalean plants studied, and that the appearance is due to slight changes in differential growth of the stamen bases which affects their relative positions. Comparable developmental studies should be made of other obdiplostemonous families, to determine the sequence of stamen initiation. For the taxa in Table 1 with numerous stamens, there s little or no doubt concerning the centrifugal order of stamen initiation.

Stamen fascicles. — Centrifugal stamens may be uniformly distributed around the receptacle as in *Dillenia* (Wilson, 1937, 1965) or they may be grouped in fascicles or clusters, as in *Candollea*. In polyandrous flowers with centripetal stamen initiation, members of the first whorl of stamens occupy positions consistent with the previous phyllotaxy of the flower. Subsequently initiated stamens however usually arise in a sequence alien to the established phyllotaxy. These later-formed stamens arise in positions below but to either side of the first stamen, so that they are not radially arranged. This sequence has been shown for *Hypericum* (Leins, 1964b), *Paeonia* (Hiepko, 1965), *Hibbertia* (Sattler, 1968), *Saurauja* (Brown, 1935), *Punica* (Mayr, 1969), *Pereskia* (Boke, 1963), *Limnocharis*, and *Hydrocleis* (Kaul, 1967, 1968). Corner stressed that the floral apical meristem ceases to act as the control centre for organ formation in such flowers after the initial stamen whorl. If order exists in each stamen fascicle, it centres around each of the first stamens (Leins, 1964a).

Of the many families with centrifugal stamens, 14 in Table 1 are reported to show fascicled arrangement of stamens. Eames (1961) says that such fascicles show varying degrees of fusion of the filaments, including occasional free or solitary stamens. Eames's use of the term fascicle is broad, since it includes single stamens with more than one trace. Eames uses the variability in degree of fusion among the stamen filaments as evidence that fascicles represent fusion products of previously separate stamens. Another viewpoint (discussed also in Eames, 1961, and in Puri, 1951) suggests that stamen fascicles have evolved by subdivision of one stamen to form many (chorisis or dé doublement). Fascicles also have been interpreted as possible branch systems or telomes (Wilson, 1937) or as the fertile portion of leaf-plus-axis systems (Melville

TABLE 1

Order	Family	Genera Studied with Centrifugal Stamens	Authority for Centrifugal Stamens	Authority for Stamen Fascicles	Authority for Stamen Trunk Bundles
DILLENIALES:					
1.	Crossosomataceae	*Crossosoma*	Payer, Corner	....	Wilson (1937)
2.	Dilleniaceae	*Hibbertia, Tetracera, Candollea*	Payer, Corner, Dickison (1970)	Wilson (1937. *Hibbertia*) Corner (*Hibbertia, Candollea*)	Wilson (1937: *Wormia, Dillenia, Doliocarpus*) Wilson (1965: *Hibbertia*) Sporne (1958: *Hibbertia*)
3.	Paeoniaceae	*Paeonia*	Hiepko, Schöffel (1932)	Hiepko, Schöffel	Sporne (1958), Brouland (1935)
MYRTALES:					
4.	Lythraceae	*Lagerstroemia, Lythrum*	Mayr; Sattler and Cheung; Payer (equivocal for *Lythrum*)	....	....
5.	Myrtaceae	*Melaleuca*	Leins (1965) (equivocal)	....	....
6.	Punicaceae	*Punica*	Payer; Mayr	....	....
SAPINDALES:					
7.	Rutaceae	*Citrus*	Corner	....	....
8.	Zygophyllaceae	*Peganum, Nitraria*	Corner; Payer (centrifugal obdiplostemony)	....	....
GERANIALES:					
9.	Geraniaceae	*Monsunia, Geranium, Pelargonium*	Payer (equivocal) Corner (centrifugal obdiplostemony, but also some taxa centripetal)	....	....
10.	Oxalidaceae	*Oxalis*	Payer (centrifugal obdiplostemony, but centripetaly also occurs in family)	....	....
CARYOPHYLLALES:					
11.	Aizoaceae	*Mesembryanthemum*	Payer	Payer	Sporne (1958)
12.	Caryophyllaceae	*Cerastium, Malachium*	Payer (2 whorls only)	....	....
13.	Portulacaceae	*Portulaca*	Payer (but equivocal) cited by Corner	....	....
CACTALES:					
14.	Cactaceae	*Opuntia, Pereskia*	Payer, cited by Corner	....	Boke (1963: *Pereskia*)

GUTTIFERALES:					
15.	Actinidiaceae	*Actinidia, Saurauja*	Brown, Corner	Brown (*Saurauja*)	Sporne (1958)
16.	Guttiferae	....	Corner	Wilson (1937: *Vismea, Symphonia*); Kawano (4 genera)	Wilson (1937: *Symphonia*) Kawano (1965: 4 genera)
17.	Hypericaceae	*Brathys, Hypericum*	Corner, Hirmer, Leins (1964a), Payer, Sporne	Corner, Hirmer, Sporne (*Hypericum*)	Saunders (1936b), Sporne (1958)
18.	Ochnaceae	....	Corner	....	Wilson (1937: *Ouratea, Ochna, Cespedesia*
19.	Theaceae	*Gordonia, Thea, Visnea*	Corner, Payer	Brown (*Gordonia, Kielmeyera*)	Sporne (1958)
LECYTHIDALES:					
20.	Lecythidaceae	*Couroupita*	Hirmer, Thompson (1927)	....	Sporne (1958)
MALVALES:					
21.	Bombacaceae	*Bombax, Hampea*	....	Wilson (1937, *Bombax*)	Wilson (1937: *Bombax, Hampea*)
22.	Malvaceae	*Hibiscus, Lavatera, Malvaviscus*	Payer	Wilson (1937), van Heel	Wilson (1937, *Hibiscus, Pavonia*): van Heel (1966)
23.	Sterculiaceae	....	....	Hirmer (*Dombeya*)	Rao (1949, *Pterospermum*)
24.	Tiliaceae	*Corchorus, Tilia, Sparmannia*	Payer, Hirmer, van Heel (1966)	Hirmer (*Sparmannia*)	Wilson (1937),Sporne (1948, 1958: *Sparmannia*)
RHOEADALES:					
25.	Capparidaceae	*Capparis*	Payer, Corner (2 stamen whorls only)	....	....
BIXALES:					
26.	Bixaceae	*Bixa*	Corner	....	Wilson (1937, *Bixa*)
27.	Cistaceae	*Cistus, Helianthemum*	Hirmer, Corner (centrifugal obdiplostemony)	....	Saunders (1936a)
28.	Cochlospermaceae	....	....	....	Wilson (1937, *Cochlospermum*)
29.	Flacourtiaceae	....	Corner	Wilson (1937, *Lindackeria*)	Wilson (1937), Sporne (1958 for *Lindackeria*)
30.	Loasaceae	*Bartonia, Cajophora, Mentzelia*	Payer, Hirmer, Corner	Hirmer (*Loasa, Blumenbachia*)	Sporne (1958)
ALISMALES:					
31.	Butomaceae	*Hydrocleis, Limnocharis*	Kaul (1967, 1968)	absent (Kaul)	Kaul (1967)
COMMELINALES:					
32.	Commelinaceae	*Tradescantia*	Payer (equivocal); Rohweder calls it centripetal	....	....

1962, 1963). In his definitive study of the Dilleniaceous genus *Hibbertia*, Wilson (1965) suggests that the fasciculate stamen arrangement and associated characteristics represent an ancient habit, long-established in the family, the origins of which are now obscured.

Stamen trunk bundles.—The vasculature of stamen fascicles is unusual in that one trunk bundle in the receptacle supplies all the stamens of the fascicle, and no other floral appendages; such flowers usually have between 3 and 20 trunk bundles. This condition, reported in 22 families (Table 1), contrasts the vasculature of most polymerous flowers in which trunk bundles supply different kinds of appendages in one sector, and in which trunk bundles are interconnected to some extent. Among the families having stamen fascicles, there are dendritic trunk bundles supplying each fascicle in *Sparmannia* (Sporne, 1948), *Hibbertia*, *Hypericum*, *Mesembryanthemum*, and *Paeonia* (Sporne, 1958). Trunk bundles are present in numerous Dilleniaceous taxa (Wilson, 1937, 1965); in members of Crossosomataceae, Guttiferae, Ochnaceae, Bombacaceae, Malvaceae, Tiliaceae, Cochlospermaceae, Bixaceae, Flacourtiaceae (Wilson, 1937). Stamen trunk bundles occur in only one monocotyledonous family, Butomaceae (Kaul, 1967, 1968), as far as is known, and the stamens there are not fascicled. Van Heel (1966, 1969) found stamen trunk bundles in several Malvalean taxa, in which stamens are usually connate in a tube rather than fascicled. In Wilson (1937) the stamen fascicletrace may supply some bundles to sepals, petals, or carpels, but generally the plants in Table 1 have discrete stamen trunk bundles. The term also is used by Eames (1961) for stamen traces if the stamen has more than a single bundle, on the premise that the extra vascular strands represent traces of lost members. Therefore, reports of stamen fascicle bundles should be examined carefully before making comparisons.

In a comprehensive survey of 140 species of *Hibbertia*, Wilson (1965) describes 5 to 15 stamen trunk bundles per flower. The most primitive taxa have many trunk bundles, while more advanced taxa have either fewer trunk bundles, or each trunk bundle supplies a single stamen. With evolutionary advancement in the genus, the number of stamen trunk bundles has been reduced, and there are fewer branches per trunk. Wilson suggests that the stamen trunk bundle is an ancient feature, and that "stages in its formation are no longer evident". Curiously, the stamen trunk bundles often arise at different levels in the receptacular vascular cylinder in *Hibbertia*.

Another unusual feature of stamen trunk bundles is their orientation of xylem and phloem; rather than being collateral, trunk bundles of many genera of different families are reported (Wilson, 1937; Camp and Hubbard, 1963) to have amphicribral, protostelic, or even siphonostelic arrangement. These complex vascular patterns contrast those of Ranalian floral receptacles, in which most traces are collateral. The unusual histological arrangements in the trunk bundles, although little is known about them, may be correlated with centrifugal or delayed ontogeny.

The direction of vascularization in stamen trunk bundles is another subject about which little is known. It is of interest because detached axillary meristems in vegetative shoots are vascularized initially by discontinuous procambium, which later becomes continuous with the axial vascular cylinder by late bidirectional differentiation. Three studies provide some information on direction of vascularization: Kaul (1967, 1968) mentions that stamen trunk bundles differentiate acropetally and continuously in *Hydrocleis* and *Limnocharis*, both of which have centrifugal initiation of stamens. Cheung and Sattler (1967) report a similar pattern of procambialization in the androecium of the flower of *Lythrum*; it has centrifugal initiation of stamen whorls, but there are only two whorls and no trunk bundles supply the stamens. My own investigations of *Hibbertia* (unpublished) show acropetally continuous procambial strands supplying the stamens as they are initiated in centrifugal order. More evidence is needed on direction of procambialization in flowers, particularly those possessing stamen fascicles and/or centrifugal pattern of androecial initiation.

It is proposed that there is an ontogenetic basis for the complex of associated characteristics including centrifugal stamen initiation, fasciculate arrangement of stamens, discrete stamen-trunk bundles, and possibly the anomolous histological structure of the trunk bundles. Melville (1963) concludes that centrifugality and its associated features cast doubt on the classical interpretation of the flower as a modified shoot bearing appendages, because vegetative shoots show no comparable patterns of development. I suggest that a more constructive approach would be to analyze the developmental basis for this assemblage of features.

Vegetative shoots have structures comparable (but not homologous) to centrifugally initiated stamens; delayed axillary bud meristems, which are undifferentiated regions surrounded by mature tissue. Axillary meristems also differ from other portions of the vegetative body in that they may be initially unvascularized. In such buds, the first leaf traces of the axillary bud initiate at the base of the bud, extend downward, and connect with the main vascular cylinder on either side of the leaf gap, while remaining quite separate from nearby subtending leaf traces.

Some aspects of this vegetative ontogeny resemble development in centrifugal androecia. The earliest stamens arise in a whorl in the usual acropetal sequence of appendages, each supplied by an acropetally developing procambial trace. Later-formed stamens however are initiated in a pocket or enclave of meristematic tissue, surrounded both adaxially and abaxially by differentiating tissue. Associated stamen traces differentiate also within a restricted area of meristem, surrounded by more mature tissues. By way of contrast, centripetally initiating flowers such as *Drimys winteri* (Tucker, 1959) show initiation of the stamen primordia on the periphery of a meristematic dome, and their procambial traces differentiate within essentially meristematic tissues. Stamens are vascularized by acropetally developing traces derived directly from the vascular cylinder.

In centrifugally initiating androecia, it is suggested that the restricted region of meristem available inhibits the pattern of vascularization. Rather than arising from the receptacular vascular cylinder, procambial traces are derived from the trunk bundles supplying the first stamens, which are the youngest primordia in the vicinity. The number of branches supplied by the trunk bundle depends on the duration of the androecial meristem and the number of stamens initiated. The enclaves of meristem left behind by the floral apical meristem are analogous to axillary meristems left behind by the terminal vegetative meristem, in that the terminal meristem no longer exerts strict control in phyllotaxy and vascularization. In a flower which develops centrifugally, with meristematic enclaves, dendritic trunk bundles would be a predictable form of vascular supply, particularly if numerous stamens are produced.

The ontogenetic basis is more puzzling for fasciculate arrangement of stamens, and for amphicribral or protostelic bundles supplying the stamens. Fascicles would result if subsequent stamens form only adjacent to each of the first-formed stamens, as clusters around them. In other words, stamen primordia would be limited to certain sectors of the cylindrical androecial meristem. Many of the taxa mentioned in Table 1 have stamens distributed uniformly around the receptacle rather than in sectors. It is possible that the difference between these two conditions lies in the total number of stamens; if they become very numerous, original boundaries between clusters would become obscured unless the flowers also have stamen trunk bundles to indicate limits of a fascicle.

Structure and function. — The roles of the flower as a pollination device and as a seed dispenser should be kept in mind in comparative morphological studies. Some excellent examples in which structure is correlated with observed function include those by Berg (1966, 1969) on seed and fruit biology, Carr and Carr (1961) on function of syncarpy, and Grant (1950) on ovule protection from pollinators. Stebbins (1967) and Carlquist (1969) suggest re-examination of prevailing assumptions about the flower, such as the idea that evolution moves only toward reduction or loss, and toward smaller size or fewer parts. Another tenet that might be questioned is that floral characteristics are more stable adaptively than vegetative characteristics. Many floral features undoubtedly have gone through unstable periods. Straw (1956) shows that features of color, nectar location, and shape and size of corolla have evolved in *Penstemon* in response to the type of pollinator. Variability of the gynoecium among Magnoliaceae provides another example of past instability of floral features at some time during generic evolution. Floral diversity is greater in Magnoliaceae than vegetative diversity; taxa are relatively homogeneous in vegetative characteristics so that sterile herbarium specimens are often difficult to identify even to genus. Trends in the magnoliaceous gynoecium (Dandy, 1927; Canright, 1960) have been toward concrescence of carpels (*Alcimandra, Aromadendron, Pachylarnax, Talauma*); toward gynophore formation (*Michelia* and section Gynopodium of *Magnolia*); toward fleshiness in *Aromadendron*, and unisexuality in *Kmeria*. Fruit dehiscence trends have been toward dorsal dehiscence in *Magnolia, Manglietia, Michelia, Alcimandra,* and *Pachylarnax*; toward ventral plus dorsal dehiscence in *Kmeria*, and toward indehiscence in *Aromadendron* and *Liriodendron*. If floral characteristics have undergone adaptive evolution in the past, it is reasonable to assume that these characteristics can still change with shifts in adaptive pressure.

Vascular ontogeny. — Complex vascular systems, such as those in Magnoliaceae, are best studied in young buds while the vasculature is still simple. A comparison of the treatments by Melville (1969), and by Skipworth (1970) and Skipworth and Philipson (1966) graphically illustrates the advantages of seeing young material. Skipworth and Philipson show ontogeny of appendages and vasculature to be closely interdependent. Puzzling features of the "double" vascular cylinder of the flower are simplified by studying an ontogenetic series. Skipworth and Philipson show that dorsal carpellary bundles diverge from the inner cylinder, which is the first portion of the vasculature to be differentiated at the level of the gynoecium. The ventral carpellary bundles diverge later in ontogeny, at a time when the outer vascular cylinder has differentiated in the receptacle. In *Michelia figo* (Tucker, 1961), a member of Magnoliaceae, I found that a time interval of 15 plastochrons separates differentiation of the dorsal and ventral strands in one carpel. Events such as ovule initiation occur concurrently with development of certain bundles, but widely separated temporally from development of other bundles. The dorsal bundle in *Magnolia* and *Michelia* species differentiates within the Carpel long before ovules form (personal observations). Vasculature of the carpel is much more complex when ovules are initiated, and they usually are vascularized by bundles other than the dorsal.

Carlquist (1969) suggests that the unusually abundant vasculature such as that of magnoliaceous carpels and receptacles is correlated with large size. One might add that long duration of the flower and fruit also may have given an adaptive advantage to ample vasculature. This possibility is supported by the initial simplicity in young flowers and the complexity which develops with time and size increase. As Camp and Hubbard (1963) show for *Paeonia*, early stages of carpel vascularization are relatively simple, but later become extremely complex.

Ovular position and vascular supply.— Study of ontogenetic stages clarifies questions involving the position of ovules on the carpel wall. Leinfellner (1965, 1966) reports some ovules are located in the ventral median position in carpels of *Drimys*, based on his study of transverse sections of large carpels. I have examined quantities of cleared and sectioned carpels of several species of *Drimys* and have failed to find ovules in this position in young carpels at the time ovules are being initiated, or while they are small. While older carpels do show ovules attached in ventral median position above or below the cleft, their absence at younger stages suggests that secondary shifts in ovular position, carpel enlargement, and differential growth in intervening portions of the carpel wall are responsible for apparent median position of ovules.

Leinfellner (1965, 1966) also reported medianly positioned ventral bundles in some species of *Drimys*, again based on trans-sections of large carpels. The median ventral strand was also used as evidence of peltation in *Drimys* carpels. The ascidial growth pattern has been shown elsewhere for *D. lanceolata* (Tucker and Gifford, 1966b), in which distribution of meristem in the carpel shows how the ascidial or modified peltate form arises. Examination of cleared carpels of *Drimy winteri* and *D. lanceolata* (personal observation) supports Leinfellner's contention, at least to the point that the lowermost ovular trace is derived from a late-forming strand running diagonally between adjacent ventral bundles. Vascularization of ovules in *Drimys* is to be discussed at more length in a later publication.

The usual derivation of ovular bundles from the ventral rather than dorsal bundle is used by Melville (1962, 1963) as evidence that the carpel is a dual structure comprised of a sterile blade and a fertile portion. The ovular supply however can be more simply explained in terms of ontogenetic timing, as previously described for *Magno-*

lia and *Michelia.* Concurrence between time of ovule initiation and time of ventral bundle differentiation probably determines the usual source of ovular vascular supply.

In the few documented examples of ovular supply from the dorsal bundle, there has been little speculation regarding why it should differ from the common type. A possible suggestion can be advanced for dorsal bundle participation in ovule supply in *Drimys lanceolata* (Tucker and Gifford, 1964, 1966b). There is only a short temporal interval between differentiation of the dorsal and the ventral bundles in this flower, and both are present and producing branches at the time of ovule initiation. Canright (1960) showed another case in which the lowermost carpels of some magnoliaceous gynoecia are vascularized jointly by ventral and dorsal bundle branches. Richardson (1969) showed at least 10 variations in the way ovules are vascularized in *Brasenia* (Nymphaeaceae). Ontogenetic studies of these and other exceptions to the usual pattern of ovule vascular supply might show atypically concurrent development of dorsal and ventral bundles in these exceptions.

CONCLUSIONS

Morphological problems particularly in need of ontogenetic study include direction and pattern of vascularization of centrifugal stamen fascicles; functional significance of concentric versus collateral stamen bundles; and relationship between obdiplostemonous members and polystemonous members, both showing centrifugal androecia. Another intriguing problem would be a comparative developmental study of centrifugal and centripetal members in one family, such as Dilleniaceae.

REFERENCES

BERG, R. Y. 1966. Seed dispersal of *Dendromecon*: its ecologic, evolutionary, and taxonomic significance. *Am. J. Bot.* **53:** 61–73.

——. 1969. Adaptation and evolution in *Dicentra* (Fumariaceae) with special reference to seed, fruit, and dispersal mechanism Nytt. *Bot. Mag.* **16:** 49–75.

BOKE, N. H. 1963. Anatomy and development of the flower and fruit of *Pereskia pititache. Am. J. Bot.* **50:** 843–858.

BROULAND, M. 1935. Recherches sur l'anatomie florale des Renonculacèes. *Le Botaniste* **27:** 1–252.

BROWN, E. G. S. 1935. The floral mechanism of *Saurauja subspinosa. Trans. Bot. Soc. Edinb.*, **31:** 485–497.

CAMP, W. H., AND M. M. HUBBARD. 1963. Vascular supply of the ovule and aril in peony and of the aril in nutmeg. *Am. J. Bot.* **50:** 171–178.

CANRIGHT, J. E. 1960. The comparative morphology and relationships of the Magnoliaceae. III. Carpels. *Am. J. Bot.* **47:** 145–155.

CARLQUIST, S. 1969. Toward acceptable evolutionary interpretations of floral anatomy. *Phytomorphology* **19:** 332–362.

CARR, S. G. M., AND D. J. CARR. 1961. The functional significance of syncarpy. *Phytomorphology* **21:** 249–256.

CHEUNG, M., AND R. SATTLER. 1967. Early floral development of *Lythrum salicaria. Can. J. Bot.* **45:** 1609–1618.

CORNER, E.J.H. 1946. Centrifugal stamens. *J. Arnold Arbor.* **27:** 423–437.

DANDY, J. E. 1927. The genera of Magnolieae. *Kew Bull.* **1928:** 257–264.

DICKISON, W. C. 1970. Comparative morphological studies in Dilleniaceae. VI. Stamens and young stem. *J. Arnold Arbor.* **51:** 403–418.

EAMES, A. J. 1961. *Morphology of the Angiosperms.* McGraw Hill, N.Y.

GRANT, V. 1950. The protection of the ovules in the flowering plants. *Evolution* **4:** 179–201.

HEEL, W. A. VAN. 1966. Morphology of the androecium in Malvales. *Blumea* **13:** 177–394.

——. 1969. The synangial nature of pollen sacs on the strength of congenital fusion and "conservatism of the vascular bundle system" with special reference to some Malvales. *Proc. K. nederl. Akad. Wet. Ser. C.* **72:** 173–206.

HIEPKO, P. 1965. Das zentrifugale Androecium der Paeoniaceae. *Deut. Bot. Gesells. Ber.* **77:** 427–435.

HIRMER, M. 1918. Beiträge zur Morphologie der polyandrische Blüten. *Flora* **110:** 140-192.

Kaul,R. B. 1967. Ontogeny and anatomy of the flower of *Limnocharis flava* (Butomaceae). *Am. J. Bot.* **54:** 1223–1230.

——. 1968. Floral development and vasculature in *Hydrocleis nymphoides* (Butomaceae). *Am. J. Bot.* **55:** 236–242.

Kawano, S. 1965. Anatomical studies on the androecia of some members of the Guttiferae-Moronoboideae. *Bot. Mag., Tokyo* **78:** 97-108.

Leinfellner, W. 1965. Wie sind die Winteraceae-Karpelle tatsächlich gebaut ? I. Die Karpelle von *Drimys*. Sektion Tasmannia. *Öst. bot. Z.* **112:** 554–575.

——. 1966. Wie sind die Winteraceae-Karpelle tatsächlich gebaut ? II. Über das Vorkommen einer ringformigen Plazenta in der Karpellen von *Drimys*: Sektion Wintera. *Öst. bot. Z.* **113:** 84–95.

Leins, P. 1964a. Das zentripetale and und zentrifugale Androecium. *Deut. Bot. Gesells Ber.* **77:** 22–26.

——. 1964b. Die frühe Blütenentwicklung von *Hypericum hookerianum* and H. *aegypticum*. *Deut. Bot. Gesells Ber.* **77:** 112–123.

——. 1964c. Entwicklungsgeschichtliche Studien an Ericales-Blüten. *Bot. Jb.* **83:** 57–88.

—— 1965. Die Infloreszenz and frühe Blütenentwicklung von *Melaleuca nesophila* (Myrtaceae). *Planta* **65:** 195–204.

Mayr, B. 1969. Ontogenetische Studien an Myrtales-Blüten. *Bot. Jb.* **89:** 210–271.

Melville, R. 1962. A new theory of the Angiosperm flower. I. The gynoecium *Kew Bull.* **16:** 1-50.

——. 1963. A new theory of the Angiosperm flower. II The androecium. *Kew Bull.* **17:**1-63.

——. 1969. Studies in floral structure and evolution. I. The Magnoliales. *Kew Bull.* **23:** 133-180.

Payer, J.B. 1857. *Traité d'organogénie comparée de la fleur*. Vols I, II. Paris.

Puri, V. 1951. The role of floral anatomy in the solution of morphological problems. *Bot. Rev.* **17:** 471–553.

Rao, C. V. 1949. Floral anatomy of some Sterculiaceae with special reference to the position of the stamen. *J. Indian bot. Soc* **28:** 237–245.

Richardson, F. C. 1969. Morphological studies of the Nymphaeaceae. IV. Structure and development of the flower of *Brasenia schreberi*. *Univ. Cal. Publ. Bot.* **47:** 1–45.

Rohweder, O. 1963. Anatomische und histogenetische Untersuchungen an Laubsprossen der Commelinaceen. *Bot. Jb.* **82:** 1–99.

Sattler, R. 1968. Centrifugal stamen development in *Hibbertia scandens*. *Am. J. Bot.* **55:** 702.

Saunders, E. R. 1936a. The vascular ground-plan as a guide to the floral ground-plan: illustrated from Cistaceae. *New Phytol.* **35:** 47–67.

——. 1936b. On rhythmic development and radial organisation in the flower. *J. Linn. Soc. Bot.* **50:** 291–322.

Schöffel, K. 1932. Untersuchungen über die Blütenbau der Ranunculaceen. *Planta, Arch. wiss. Bot.* **17:** 342–344

Skipworth, J. P. 1970. Development of floral vasculature in the Magnoliaceae. *Phytomorphology* **20:** 228–235.

Skipworth, J. P., and W. R. Philipson. 1966. The cortical vascular system and the interpretation of the *Magnolia* flower. *Phytomorphology* **16:** 463–468.

Sporne, K. R. 1948. A note on a rapid clearing technique of wide application. *New Phytol.* **47:** 291–2.

——. 1958. Some aspects of floral vascular systems. *Proc. Linn. Soc. London,* **169:** 75–84.

Stebbins, G. L. 1967. Adaptive radiation and trends of evolution in higher plants. In *Evolutionary Biology*. T. Dobzhansky, M.K. Hecht, and W.C. Steere, (eds.) Vol. 1, N.Y. 101–142.

Straw, R. M. 1956. Adaptive morphology of the *Penstemon* flower. *Phytomorphology* **6:** 112–119.

Thompson, J. M. 1927. A study in advancing gigantism with staminal sterility, with special reference to Lecythidaceae. *Publ. Hartley bot. Labs. Liyverpool. Univ.* Pt. 4.

Tucker, S. C. 1959. Ontogeny of the inflorescence and the flower in *Drimys winteri* var. *chilensis*. *Univ. Cal. Publ. Bot.* **30:** 257–336.

——. 1961. Phyllotaxis and vascular organization of the carpels in *Michelia fuscata*. *Am. J. Bot.* **48:** 60–71.

——, and E.M. Gifford. 1964. Carpel vascularization of *Drimys lanceolata*. *Phytomorphology* **14:** 197–203.

——. 1966a. Organogenesis in the carpellate flower of *Drimys lanceolata*. *Am. J. Bot.* **53:** 433–442.

——. 1966b. Carpel development in *Drimys lanceolata*. *Am. J. Bot.* **53:** 671–678.

Wilson, C. L. 1937. The phylogeny of the stamen. *Am. J. Bot.* **24:** 686–699.

——. 1965. The floral anatomy of the Dilleniaceae. I. *Hibbertia*. *Phytomorphology* **15:** 248–273.

Adv. Pl. Morph. 1972: 370-375.

A NEW KIND OF ENDOSPERM IN *RHINANTHUS MAJOR* EHRH AND *R. SEROTINUS* (SCHOENL) SCHINZ AND THELL

B. TIAGI

Department of Botany, University of Rajasthan, Jaipur-4, India

ABSTRACT

The sessile, campylotropous, discoid ovule is borne on a nodular placenta. The multinucleate micropylar haustorium grows as tubular body in the micropylar canal and profusely branches in the placenta. The chalazal haustorium is two- nucleate and highly aggressive. Endosperm is differentiated into three regions—the middle storage, the haustorial micropylar, and chalazal. The latter two, are being reported as new kind of endosperm.

INTRODUCTION

Rhinanthus belongs to the subfamily Rhinanthoideae of the family Scrophulariaceae which contains many taxa which are semi-parasitic, growing in Swampy grasslands, attached to the roots of grasses. Endosperm formation in the family has received considerable attention of the embryologists (Banerji, 1961) but the new kind of micropylar and chalazal endosperm which is haustorial in function being described herein, has not been reported so far in any taxa. Tiagi (1966) described the development of the seed and fruit in *Rhinanthus* paying some attention to this secondary haustorial endosperm.

MATERIAL AND METHODS

Material of *Rhinanthus major* was collected from Leningrad, U.S.S.R. in July, 1970; and *R. serotinus* was obtained through the courtesy of the Director, University Botanical Gardens, Copenhagen. It was processed in the usual manner for embedding in paraffin-wax. Sections were cut at 10 to 15 μ and stained with safranin-fast green combination. Mature dry seeds of *R. major* were cut into two halves and soaked in water, to treat the endosperm with reagents for the test of starch, oil, and protein.

OBSERVATIONS

Ovule.—The ovule is sessile, campylotropous and laterally flattened. It is borne on a nodular placenta which has a compact core, enveloped in a spongy tissue (Fig. 1). The placental supply consisting of provascular strands is branched profusely in the nodule. The main peripheral strand, however, ends in the chalaza (Figs. 1, 7).

Endosperm.—The primary endosperm nucleus lies near the zygote. It divides transversely and partitions the embryo sac into two unequal chambers—micropylar and chalazal. The former is smaller, and the latter larger in size (Tiagi, 1966).

The chalazal chamber develops directly and immediately into a two-nucleate chalazal haustorium (Fig. 2). The micropylar chamber divides longitudinally, and transversely afterwards, to separate the two micropylar haustorial cells from the two initials of the endosperm proper (Fig. 2). The micropylar haustorium functions rather late (Figs. 3, 5) in contrast to the chalazal haustorium which becomes aggressively functional soon after its formation.

Micropylar haustorium.—The two uninucleate and vacuolate micropylar haustorial cells enclose the zygote, and fuse upwards to form a common tubular body which grows in the micropylar canal ultimately entering in the nodular placenta (Fig. 7). The haustorial tube may perforate the ovule near the micropyle if the latter is not in intimate contact with the placenta (Figs. 7, 8). The binucleate haustorium becomes multinucleate (Figs. 3, 5, 7, 9) and the nuclei hypertrophied. The haustorium branches profusely in the nodular placenta. Its rhizomycelium .like fine attenuated branches follow a course parallel to the provascular strands in the placenta (Fig. 7). The haustorum completely depletes the swollen placenta of its contents. It becomes inconspicuous (Fig. 17) but darkly stained prominent remnants of the haustorial branches are visible in it.

The micropylar haustorium before flowing in the micropylar canal may precociously give small haustorial branches in the endothelium and the integument (Figs. 1, 3). Such branches may also arise from well developed haustoria growing in the micropylar canal; the branches enter the integumentary tissue (Fig. 4).

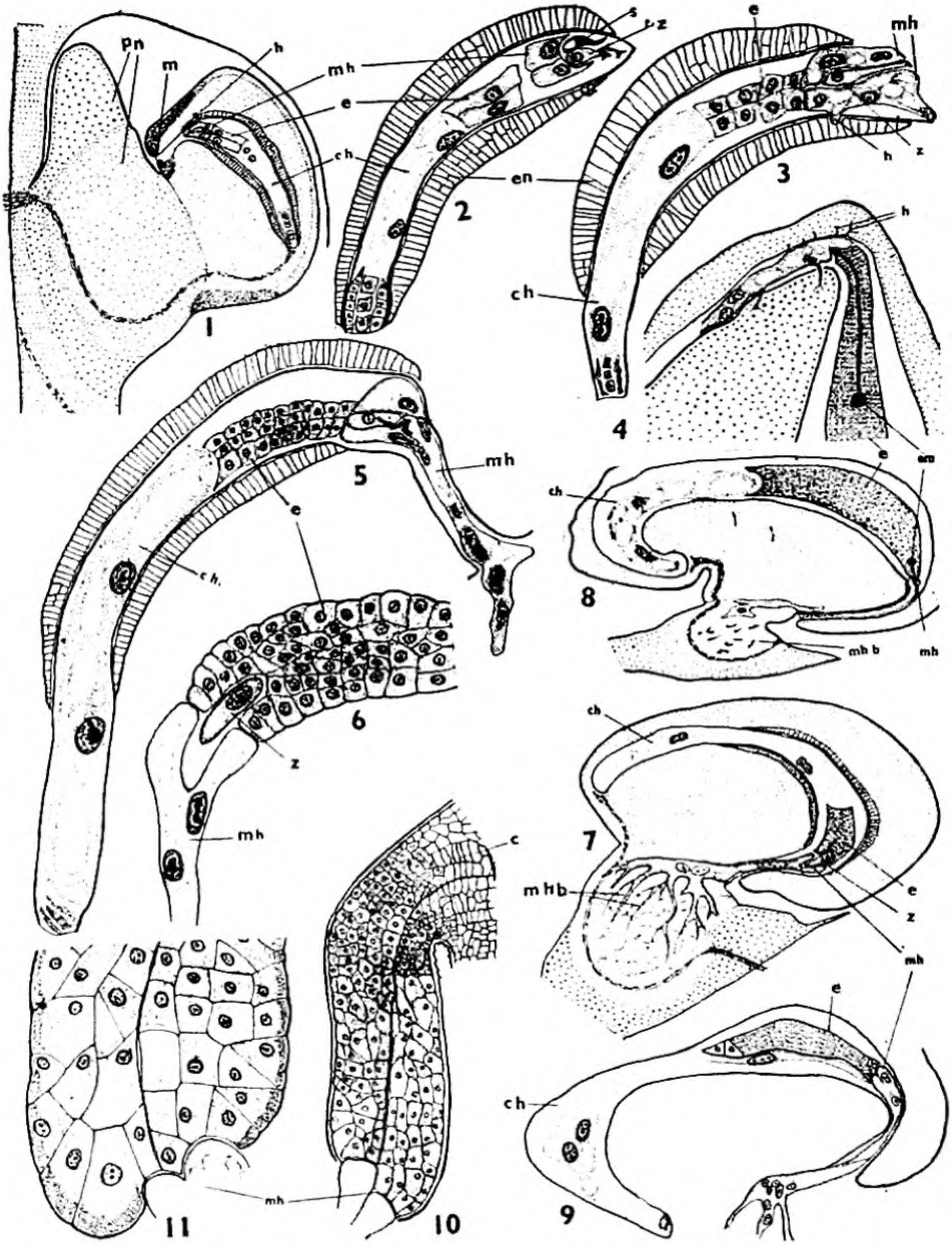
Chalazal haustorium.— The chalazal haustorium is two nucleate, tubular, and it becomes curved (Fig. 8). Its nuclei are hypertrophied. The haustorium aggressively digests the chalazal tissue and extends itself almost to the base of the young seed (Fig. 7).

As an exception, there may be more than two nuclei in the haustorium. An additional nucleus has been observed by the side of the well-advanced endosperm. It appears that in this case, the chalazal chamber has been laterally extended upto the micropylar haustorium Also, the endosperm cells at the two ends have become enlarged and they look very different from the rest of the cells of the endosperm (Fig. 9).

Micropylar and chalazal parts of the endosperm.—The endosperm is differentiated into three distinct parts—the micropylar, the middle storage, and the chalazal. Below the micropylar haustorium, there is developed a special type of endosperm which is designated here as micropylar haustorial endosperm. It occupies the curved micropylar part of the embryo sac and a portion of the micropylar canal. It originates from the storage endosperm. This endosperm, as well as the chalazal, of similar nature, develop rather late when the haustoria are ceasing to function.

These two endosperms serve a digesting and conducting function. They absorb nutritive materials from the integument, and pass it on for storage to the bulky middle region—the endosperm proper. The chalazal endosperm is better developed than the micropylar, and it is also derived from the storage endosperm.

Micropylar haustorial endosperm.— A few layers of the storage endosperm, below the micropylar haustorium become cambium-like (Fig. 10). They actively divide and form the cells which assume a haustorial function. The growth of



FIGS. 1-11. *Rhinanthus major*. Fig. 1. L.s. ovule, micropyle in contact with placenta, micropylar and chalazal haustorium, endosperm proper. Fig. 2. Same, embryo sac, zygote, synergid, micropylar and chalazal haustorium, endosperm proper. Fig. 3. Same, zygote, micropylar and chalazal haustorium, endosperm. Fig. 4. Same, a portion, embryo, endosperm, micropylar haustorial branches. Fig. 5. Same, zygote, endosperm, micropylar and chalazal haustorium. Fig. 6. Same, a portion, zygote, micropylar haustorium, endosperm. Fig. 7. L.s. ovule, zygote, micropylar haustorial branches in placenta, endosperm, chalazal haustorium. Fig. 8. Same, embryo, endosperm, haustoria. Fig. 9. Same, a portion, three nuclei in chalazal haustorium, endosperm, micropylar haustorium, endosperm, micropylar haustorium. Fig. 10. L.s. Micropylar endosperm. Fig. 11. Same, a portion.

C, Cambium; ch, chalazal haustorium; e, endosperm; em, embryo; en, endothelium; h, haustorial branch; m, micropyle; mh, microphylar haustorium; mhb, micropylar haustorial branches; pn, placental nodule; s, synergid; z, zygote,

the endosperm pushes the micropylar haustorium to same distance in the micropylar canal (Fig. 17). The endosperm so produced surrounds the suspensor of the embryo which occupies a central position. The cells away from the storage endosperm, next to the micropylar haustorium (Figs. 10, 11) are larger in size, rounded in shape, possess dense vacuolate cytoplasm, and active nuclei. The peripheral cells, in particular, abutting the integument have denser outer layer of cytoplasm (Fig. 10). The cells adjacent the storage endosperm, being freshly derived, simulate the storage endosperm. There are also intergrading zones between these two extremes. These endosperm cells are thinwalled and they do not store any reserve materials excepting tanin which gets filled in them as they degenerate later.

Chalazal haustorial endosperm.—Cells of a broad region of the storage endosperm bordering the chalazal cavity, become cambium-like and by their growth fill the large cavity with chalazal haustorial endosperm. It also shows the same growth zonations but more clearly than the micropylar endosperm. Even prior to the formation of the cambium, the endosperm cells tend to grow peripherally in the cavity (Fig. 8). The chalazal endosperm grows peripherally hanging in the chalazal cavity (Fig. 12) and then inwardly. Often a portion of the cavity is left vacant, and it may show the degenerated remnants of the chazal haustorium.

The cells of the chalazal endosperm are thin walled, densely cytoplasmic and vacuolate. Its one or two peripheral layers are minutely vacuolate and denser cytoplasmic than the rest (Fig. 12, 13). They have a rounded shape as seen in smear preparations. Ultimately the chalazal endosperm degenerates and its cells become laden with tanniferous substances; the cambial zone becomes particularly dark-black (Fig. 18). It distinctly separates the storage endosperm from the chalazal.

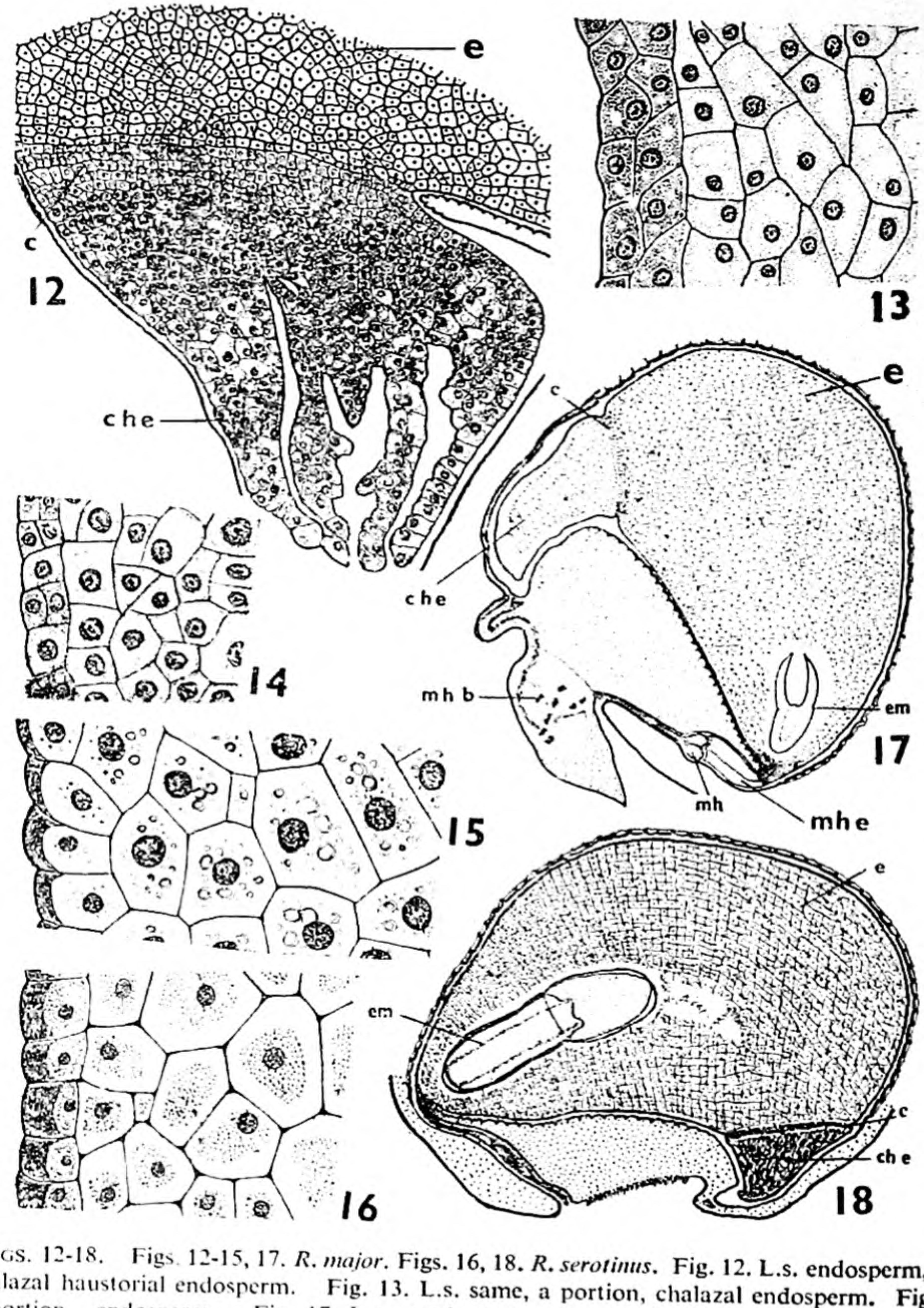
In the seed the two cavities, one at the micropylar, and the other at the chalazal end, are plugged with dead, tanniferous micropylar and chalazal endosperms respectively. The micropylar canal is often filled with remnants of the micropylar haustorial tube (Fig. 18).

Storage endosperm.—The young endosperm cells are thin walled, uninucleate and filled with vacuolate cytoplasm (Fig. 6). They divide rapidly and fill the enlarging space within the developing seed, with endosperm. Its peripheral cells forming the jacket layer also divide actively. This layer possesses denser cytoplasm, and small vacuoles (Fig. 14).

The bulk of the seed is made up of endosperm (Fig. 18). The outer tangential walls of the mature peripheral endosperm cells become thickly lamellated (Fig. 15) especially so in *R. serotinus* (Fig. 16). It forms an efficient protective pellicle over the ovoid endosperm. Cell walls of the endosperm become thickened. They store reserve food material in the form of oil droplets. Besides oil, the frothy cytoplasm of the endosperm cells becomes laden with rounded and irregular tanniferous bodies. The nucleus is reduced to a darkly staining body, embedded within the reserve materials.

DISCUSSION

In the family Scrophulariaceae, two well defined regions of the endosperm—one at the micropylar, and the other at the chalazal end are often observed (Tiagi, 1956). These originate from the endosperm proper and function during



FIGS. 12-18. Figs. 12-15, 17. *R. major*. Figs. 16, 18. *R. serotinus*. Fig. 12. L.s. endosperm, cambial zone, chalazal haustorial endosperm. Fig. 13. L.s. same, a portion, chalazal endosperm. Figs. 14-16. L.s. a portion, endosperm. Fig. 17. L.s. seed, endosperm, embryo, micropylar and chalazal endosperm, micropylar haustorial branches. Fig. 18. same, mature seed.

c, cambium; che, chalazal haustorial endosperm; e, endosperm; mh, micropylar haustorium; mhe, micropylar haustorial endosperm; mhb, micropylar haustorial branches.

the optimum activity of the micropylar and chalazal haustoria for the conduction of nutritive materials from the haustoria to the storage endosperm. After serving their function, they simply degenerate.

In *Rhinanthus*, on the other hand, the micropylar and chalazal endosperms develop when the haustoria have become somewhat inactive. Although they originate from the storage endosperm, they differ remarkably in their staining reactions, in their cellular contents, and in being haustorial. They take up the function of the two haustoria after they have ceased to function, thus compensating for their rather early inactivity during the maturation of the seed. It appears that probably a similar situation occurs in *Pedicularis* (Berg, 1954) but he remarks that the first expansion of the chalazal haustorium is directed towards the storage endosperm. The upper part of the haustorium therefore, protrudes into the endosperm like a long cone. In fact, it is not the chalazal haustorium which grows upwards into the endosperm but the endosperm that grows peripherally and later inwardly into the cavity of the chalazal haustorium.

I am grateful to Professor V. Puri for inspiring me to study for a botanical career.

REFERENCES

BANERJI, I. 1961. The endosperm in Scrophulariaceae. *J. Indian. bot. Soc.* **40:** 1-11.
BERG, R.Y. 1954. Development and dispersal of the seed of *Pedicularis silvatica* Nytt. *Mag. Bot.* **2:** 1–60.
TIAGI, B. 1956. A contribution to the embryology of *Striga orobanchoides* Benth and *S. enphrasioides* Benth. *Bull. Torrey bot. club* **83:** 154–170.
——. 1966. Development of the seed and fruit in *Rhinanthus major* and *R. serotinus*. *Am. J. Bot.* **53:** 645–651.

Adv. Pl. Morph. 1972: 376-393

ENDOSPERM IN EUPHORBIACEAE—A CRITICAL APPRAISAL

BY R. N. KAPIL AND A. K. BHATNAGAR
Department of Botany, University of Delhi, Delhi-7, India

ABSTRACT

The endosperm in Euphorbiaceae is 2 to 15-ploid. Despite this variation, there is no significant difference in the course of its development which is invariably of the Nuclear type. Only in one genus, *Croton*, an aggressive chalazal coenocytic haustorium is known. Enucleate cytoplasmic nodules occur in *Euphorbia dulcis* and *E. helioscopia* during the free nuclear stage. A characteristic feature is the synchrony in nuclear division at initial stages of development. Dixon's hypothesis suggesting the presence of a mitotic hormone and its diffusion from the micropylar to chalazal end of endosperm does not appear to hold good for *Chrozophora*, *Euphorbia* and *Securinega*.

Culture experiments with euphorbiaceous endosperm have not only revealed the potentiality of the endosperm for organogenesis like that of the embryo but also their mutual dependence in metabolic activities. Induction of root and/or shoot buds has been achieved from the triploid endosperm of *Croton bonplandianum*, *Jatropha panduraefolia* and *Putranjiva roxburghii*. In *P. roxburghii* even a complete plantlet has been obtained.

Intensive biochemical and physiological studies with endosperm of *Ricinus communis* have helped in understanding the mechanism of lipid metabolism in higher plants. Unique subcellular organelles such as glyoxysomes and proplastids have been shown to be associated with fat metabolism. There is an indication that in many species of *Euphorbia* such organelles or their contents find their way into the endosperm from nucellus.

The usefulness, importance and superiority of euphorbiaceous endosperm over others in in vivo and in vitro studies have been indicated.

INTRODUCTION

The Euphorbiaceae constitute one of the largest families among the dicotyledonous angiosperms with majority of the members distributed in tropical countries. They represent an assemblage of plants of diverse forms ranging from herbs to shrubs and trees. Almost all types of inflorescence are met with. The development and organization of the female gametophyte exhibit great diversity. As many as seven types (Polygonum, Allium, Fritillaria, Drusa, Adoxa, Acalypha indica and Peperomia hispidula) of embryo sacs have been recorded. Similarly, several types of embryogeny are known. Surprisingly, however, the development of the endosperm has remained quite conservative. Like the structure of the seed and fruit, it does not show much variability and is invariably of the Nuclear type (for literature see Thathachar, 1953a; Kapil, 1958; Davis, 1966; Bhatnagar, 1970).

Embryological and experimental studies

on euphorbiaceous endosperm have brought forth information that throws considerable light not only on the structural, developmental and cytological aspects of this tissue but also on its nature and potentialities. These studies are critically analysed in this review.

CYTOLOGY OF THE PRIMARY ENDOSPERM NUCLEUS

In the absence of actual chromosome counts the determination of ploidy status of endosperm is mostly based on the number of polar nuclei involved in the organization of the fusion nucleus. A large majority of taxa has a triploid primary endosperm nucleus but in genera such as *Acalypha* and to some extent *Euphorbia* and *Mallotus*, the ploidy level varies greatly (see Table).

In *Acalypha ciliata* (Kajale and Murthy, 1954), the location of the fusion nucleus in the embryo sac and its organization are quite peculiar. The secondary nucleus may be situated either near the egg, or in the middle or towards the chalazal end of the embryo sac (Fig. 1A-D). Occasionally all the eight polar nuclei may fuse to form an octaploid secondary nucleus (Fig. 1D), though quite frequently one of them (from the micropylar or chalazal quartet) degenerates so that only seven nuclei participate in the or-

Taxon	Investigator/s	Number of fusing polar nuclei	Nature of primary endosperm nucleus
(1) *Acalypha*			
A. australis	Tateishi (1927)	4	pentaploid
A. indica	Maheshwari and Johri (1941)	6–9	hepta- to decaploid
A. tricolor	Swamy and Balakrishna (1946)	4	pentaploid
A. rhomboidea	Landes (1946)	4	pentaploid
A. fallax	Banerji (1949)	6–9	hepta- to decaploid
A. lanceolata	Thathachar (1952)	14	15-ploid
A. ciliata	Kajale and Murthy (1954)	7–8	octa- to nonaploid
A. malabarica	Mukherjee (1958)	4	pentaploid
A. brachystachya	Kapil (1960)	4	pentaploid
A. alnifolia	Venkateswarlu and Rao (1963)	7–8	octa- to nonaploid
A. paniculata	Venkateswarlu and Rao (1963)	4	pentaploid
(2) *Euphorbia*			
E. procera	Modilewski (1909)	4	pentaploid
E. palustris	Modilewski (1911)	4	pentaploid
E. virgata	Dessiatoff (1911)	4	pentaploid
E. pulcherrima	Donati (1912)	4	pentaploid
	Sharma (1955)	2	triploid
E. dulcis	Carano (1926)	4	tetraploid
(3) *Mallotus*			
M. albus	Raju and Nagaraja Rao (1953)	2	triploid
M. japonicus	Ventura (1934)	2–4	di- to tetraploid
M. philippensis	Thathachar (1953b)	4	pentaploid

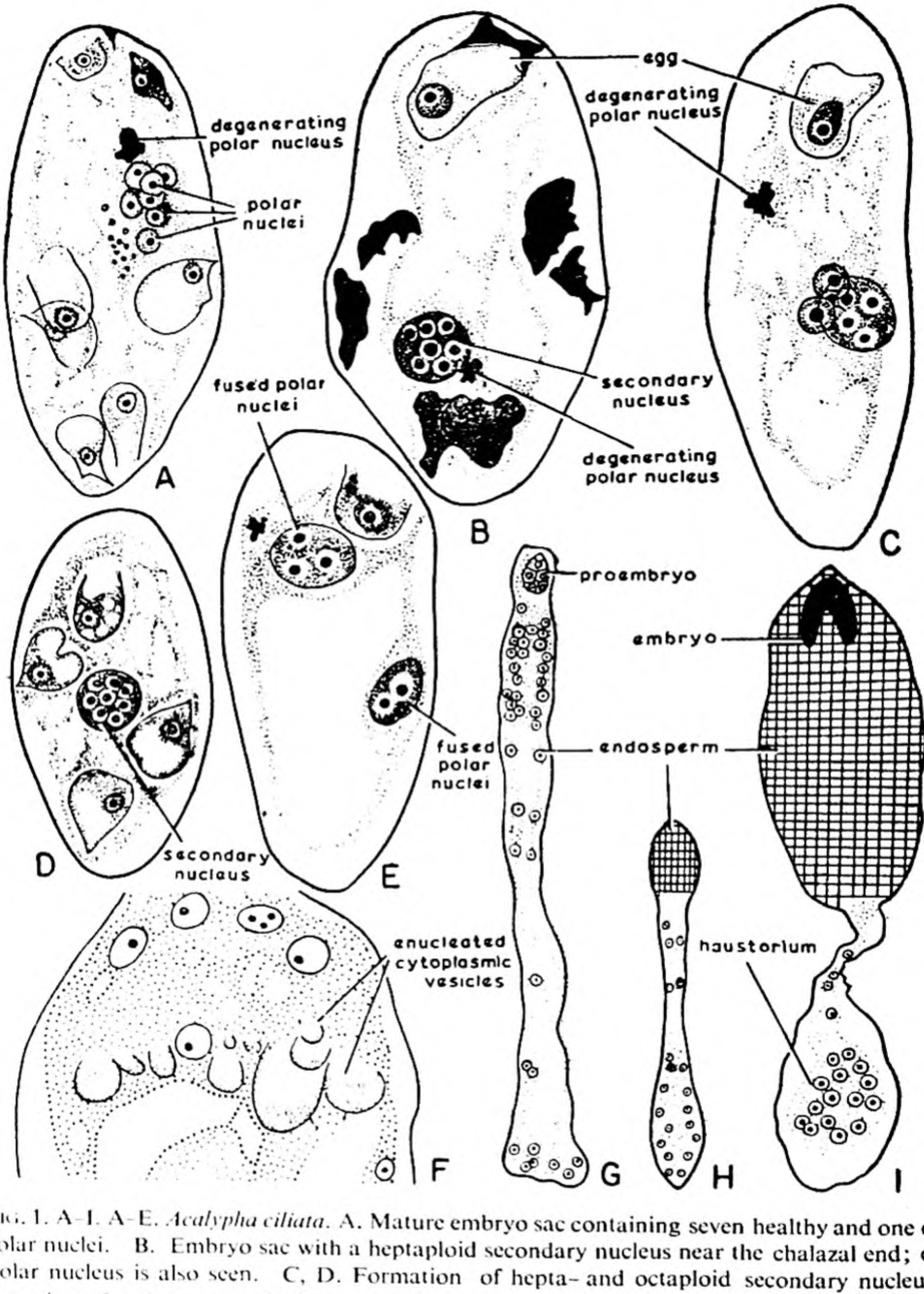

degenerating polar nucleus
polar nuclei
A
egg
degenerating polar nucleus
secondary nucleus
degenerating polar nucleus
B
C
fused polar nuclei
D
secondary nucleus
E
fused polar nuclei
proembryo
embryo
endosperm
enucleated cytoplasmic vesicles
haustorium
F
G
H
I

FIG. 1. A–I. A–E. *Acalypha ciliata*. A. Mature embryo sac containing seven healthy and one degenerating polar nuclei. B. Embryo sac with a heptaploid secondary nucleus near the chalazal end; degenerating polar nucleus is also seen. C, D. Formation of hepta- and octaploid secondary nucleus in the middle region of embryo sac. E. Polar nuclei have fused to form two distinct nuclei. F. ***Euphorbia** helioscopia*. Endosperm with enucleate cytoplasmic vesicles. G. ***Croton sparsiflorus***. Free nuclear endosperm. H. *C. klotzchianus*. Endosperm with cellular micropylar and free nuclear chalazal portions. **I. *C.*** *sparsiflorus*. Older stage showing coenocytic chalazal haustorium (A–E, after Kajale and Murthy, 1954; F, after R. P. Singh, 1969; G–I, after Venkateswarlu and Rao, 1963).

ganization of the secondary nucleus (Fig. 1A, B). The polar nuclei may even fuse in groups which may subsequently unite to produce the resultant heptaploid secondary nucleus (Fig. 1C). Rarely, the fused groups form two distinct nuclei (Fig. 1E). Whether these two nuclei represent the fusion products of two groups of polar nuclei or constitute the derivatives of the primary endosperm nucleus, needs confirmation.

In a couple of examples, autonomous development of endosperm has also been recorded. As early as 1898, Lyon found that in *Euphorbia corollata* the generative cell either does not divide at all or, if it does, one of the male cells "disappears" before the entry of the pollen tube into the micropyle and the other fertilizes the egg, implying thereby that the development of endosperm is autonomous. In *Mallotus japonicus* (Ventura, 1934) and *Euphorbia dulcis* (Carano, 1926; see also Kapil, 1961) also the endosperm develops without the participation of, or even the stimulation by, the male gamete.

TIME-LAG BETWEEN ZYGOTIC AND ENDOSPERMIC DIVISION

The primary endosperm nucleus invariably divides earlier than the zygote, except in *Breynia* (Thathachar, 1953a) where the division of the two is simultaneous. Usually 4–8 endosperm nuclei are formed when the zygote divides but the formation of as many as 32–64 endosperm nuclei is known in *Euphorbia splendens* (Weniger, 1917). The time-lag between the division of the zygote and the primary endosperm nucleus in *Aleurites fordi* (McCann, 1945) is 68 hours during which eight endosperm nuclei are formed.

Normally, the two daughter nuclei formed after the division of the primary endosperm nucleus migrate to the opposite poles of the embryo sac. Later divisions result in a large number of nuclei distributed uniformly in a parietal layer of cytoplasm (Fig. 2C). Quite frequently the nuclei aggregate around the proembryo, as in *Chrozophora rottleri, C. obliqua* (Kapil, 1956a, b) and *Aleurites fordi* (McCann, 1945). This is probably due to more intensive karyokinetic activity in the micropylar region of the embryo sac. A similar explanation has been given by McCann (1945) for *Aleurites* and Thathachar (1953a) for many other euphorbiaceous plants. Rarely, however, the accumulation of nuclei may be seen near the chalazal end as in *Acalypha indica* (Johri and Kapil, 1953). The nuclei noticed at the chalazal end of the embryo sac in *Acalypha indica* are comparatively smaller than those at the micropylar end. Formation of enucleate cytoplasmic vesicles is also recorded in *Euphorbia dulcis* (Kapil, 1961) and *E. helioscopia* (R.P. Singh, 1969; Fig. 1F).

SYNCHRONY IN NUCLEAR DIVISION

A strikingly high degree of synchrony characterizes the early nuclear divisions of the endosperm in some members of the Euphorbiaceae. In *Acalypha indica* (Johri and Kapil, 1953; Fig. 2A, B) and *A. lanceolata* (Thathachar, 1952), this may sometimes continue till at least 32 nuclei have been formed. Numerous, simultaneously dividing endosperm nuclei, have been observed in the micropylar part of the embryo sac in *Aleurites fordi* (McCann, 1945). Often, the divisions become irregular after 16–nucleate stage as in *Chrozophora obliqua* (Kapil, 1956b) or even earlier as in *Euphorbia geniculata* (Bhatnagar, 1970).

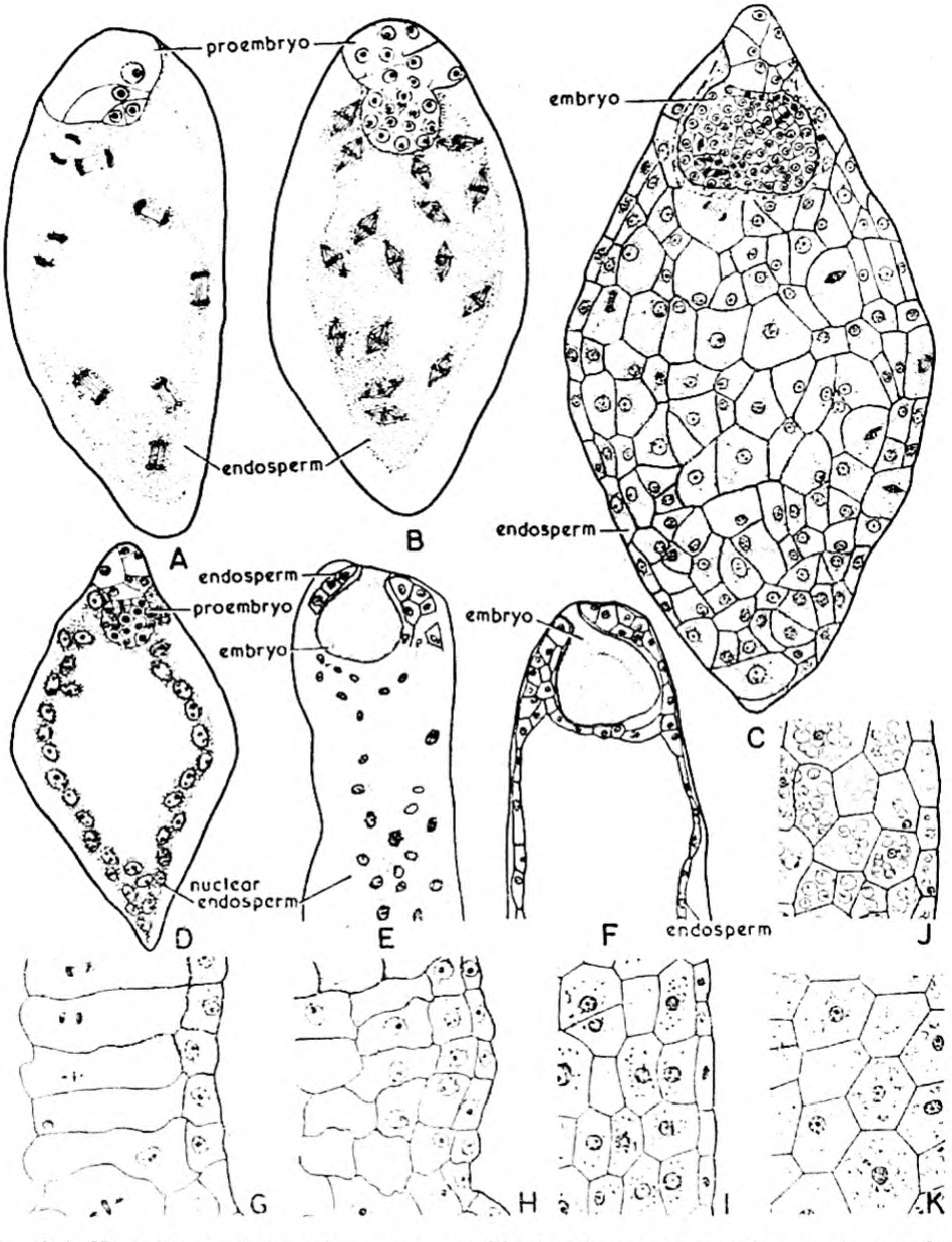

proembryo
embryo
endosperm
endosperm
A
B
C
endosperm
proembryo
embryo
embryo
nuclear endosperm
D
E
F
endosperm
J
G
H
I
K

FIG. 2 A–K. A–D. *Acalypha indica.* A, B. Fertilized embryo sacs showing synchronously dividing endosperm nuclei. C. Cellular endosperm. D. Endosperm nuclei distributed in the parietal layer of cytoplasm. E, F. *Chrozophora obliqua.* Early stages in initiation of cell wall formation from micropylar end downwards. G, H. *C. rottleri.* Portion of first formed endosperm layers undergoing repeated periclinal divisions. I. *C. obliqua.* Endosperm from a mature seed showing absence of food material in epidermal layer. J. *Acalypha brachystachya.* Endosperm from ripe seed showing presence of food reserves in epidermal layer. K. *C. rottleri.* Endosperm cells containing oil droplets. (A–D, after Johri and Kapil, 1953; E, F, after Kapil, 1956a; G–I, after Kapil, 1956b; J, after Kapil, 1960; K, after Kapil, 1956a).

Synchrony in early stages of endosperm development is also known to occur in some other dicotyledonous as well as monocotyledonous families such as Amaryllidaceae, Liliaceae, Philydraceae, Polemoniaceae, Ranunculaceae and Zosteraceae. It is, however, true that most authors do not comment on this aspect which may actually be more common than is realized (see also Erickson, 1964). As discussed elsewhere, due emphasis must be given to this feature because it makes such an endosperm a very attractive material for analytical and experimental studies on cell division.

CELL FORMATION

Cell wall formation is usually initiated at the globular stage of the proembryo. Occasionally, as in *Phyllanthus maderaspatensis* (Mukherjee and Padhye, 1964), it begins soon after the proembryo has reached the octant stage. On the other hand, in *Chrozophora rottleri*, walls are laid only after the embryo has reached the heart-shaped stage (Kapil, 1956a). Variation exists also with regard to the number of endosperm nuclei present when wall formation commences, e.g., *Acalypha lanceolata* (Thathachar, 1952) has 32, *A. indica* (Johri and Kapil, 1953) has 64 (sometimes 32), *Chrozophora* and *Euphorbia* have 100 or more and *Acalypha australis* (Tateishi, 1927) shows at least 256 nuclei.

Usually wall formation starts from the micropylar end and proceeds towards the chalazal end as in *Aleurites, Chrozophora* (Fig. 2E, F), *Euphorbia, Hevea* and *Ricinus*. However, in *Acalypha malabarica, A. ciliata* (Mukherjee, 1964) and *A. indica* (Johri and Kapil, 1953) walls are laid simultaneously along the periphery of the embryo sac. Centripetal growth of the endosperm takes place by periclinal divisions of the first formed cells and finally the entire embryo sac becomes filled with cells (Fig. 2C). In *Acalypha indica* (Johri and Kapil, 1953) and *Chrozophora rottleri* (Kapil, 1956a; Fig. 2G, H) the first formed endosperm cells divide like cambium so that tiers of radially arranged cells are formed. Cells in the centre are usually larger and much more vacuolated than those at the periphery which are narrow and elongated.

Wall formation is usually completed by the time the embryo reaches heart-shaped stage or even earlier when it is globular as in *Phyllanthus maderaspatensis* (Mukherjee and Padhye, 1964).

McCann (1945) observed that in *Aleurites fordi* the walls do not extend to the centre of the embryo sac "leaving it open", permitting expansion in length of the cotyledons of the embryo. Excepting coconut, such a situation has perhaps not been recorded in other angiosperms and needs confirmation. McCann's illustration showing the absence of walls in the middle of the embryo sac perhaps represents a transition stage. Attention may also be drawn to Srivastava and Agarwal's (1953) statement that in *Chrozophora rottleri* the endosperm remains free nuclear throughout. This is incorrect (Kapil, 1956a), probably the authors did not examine material of sufficiently advanced stage.

In *Euphorbia helioscopia* and *E. platyphylla*, Schürhoff (1926) reported that one of the synergids produces an additional embryo. This is not true, at least for *E. helioscopia* (Kapil, 1958) in which the aggregation of free endosperm nuclei around the proembryo and initiation of wall formation in this region gives a false impression of the presence of a synergid embryo.

ENDOSPERM HAUSTORIUM

Of the large number of taxa so far investigated, three species of *Croton*—*C. sparsiflorus* (=*C. bonplandianum*), *C. klotzchianus* (Venkateswarlu and Rao, 1963) and *C. oblongifolius* (Pal, 1972)—are known to form a free nuclear chalazal haustorium. In *C. sparsiflorus* and *C. klotzchianus* the haustorium is initiated as a small protrusion at four or eight-nucleate stage and gradually develops into an elongated structure. Simultaneously, free nuclear divisions continue and the nuclei become dispersed peripherally with greater accumulation in the micropylar region. Cell wall formation is restricted to the micropylar part of the embryo sac whereas the chalazal portion not only remains free nuclear throughout but also assumes the shape of a narrow tubular caecum broadening conspicuously at the chalazal end (Fig. 1G—I). This structure sometimes attains the length of 1000 μ and behaves as an aggressive chalazal haustorium. In *C. oblongifolius* (Pal, 1972) the haustorium disappears in the mature seed but in *C. bonplandianum* (R.P. Singh and Chopra, 1970) it persists even in the mature seed as a sac-like structure with degenerated nuclei. It may be recalled that in *Euphorbia procera* Modilewski (1909) noticed that the endosperm nuclei at the base of the embryo sac are larger and he attributed haustorial role to them.

Occasionally, in some species of *Euphorbia* the endosperm cells in the chalazal region, just above the hypostase, are larger and take a denser stain. According to Thathachar (1953a) these cells presumably perform a conducting role, taking up nutrition from the compact cells below (hypostase) and carrying it to the cells above. In *Acalypha brachystachya* (Kapil, 1960), the endosperm cells at the chalazal end often contain hypertrophied nuclei.

ENDOSPERM IN MATURE SEED

By the time cell formation in the endosperm is complete, major part of the nucellus is absorbed. In a mature seed only a whip-like structure, representing the crushed remnants of the nucellar beak, may sometimes be discernible at the micropylar end. In the chalazal region, however, few or several layers of nucellus may persist as perisperm as in *Acalypha, Chrozophora* and *Euphorbia* (Kajale, 1954; Mukherjee, 1957; R.P. Singh, 1969). In *Aleurites fordi* (McCann, 1945), *Codiaeum variegatum* (R.P. Singh, 1965) and *Ricinus communis* (R.P. Singh, 1954) the chalazal region of the nucellus too is encroached upon and absorbed by the enlarging endosperm.

According to Muzik (1954), in *Hevea brasiliensis*, during growth "the endosperm was absorbed by the nucellus and the major portion of the mature fleshy seed was composed of this tissue". It appears quite improbable that during the maturation of the seed the nucellus absorbs the endosperm. If so, this would constitute a unique example since normally it is the endosperm which, if at all, is dependent on the nucellus for its food material. A.N. Rao (1964) states that "most of the endosperm is utilized during the embryo development" but he does not mention anything about the fate of the nucellus.

A portion of the endosperm is consumed by the growing embryo but its major part persists so that the ripe seeds of this family are invariably albuminous. It is only in *Hevea brasiliensis* that most of the endosperm is utilized during the emryo development (A.N. Rao, 1964).

MAJOR FOOD RESERVES

The endosperm is rich in food reserves such as starch (e.g., *Acalypha malabarica*, Mukherjee, 1964; *Chrozophora obliqua*, Kapil, 1956b, Fig 2I; *Euphorbia hypericifolia*, Mukherjee, 1957; *Hevea brasiliensis*, A.N. Rao, 1964), fat globules (*Acalypha brachystachya*, Kapil, 1960; *A. malabarica*, Mukherjee, 1964; *Chrozophora rottleri*, Kapil, 1956a, Fig. 2K; *Hevea brasiliensis*, A.N. Rao, 1964; *Ricinus communis*, R.P. Singh, 1954) and proteins (*Ricinus communis*, Canvin, 1963). Most of the investigators have paid little attention to the qualitative as well as quantitative analyses of the food reserves in the mature endosperm and this aspect deserves further study.

In *Acalypha brachystachya*, the epidermal layer of endosperm, like the other cells, shows the presence of reserve food materials (Fig. 2J). In *A. rhomboidea* and *Euphorbia maculata* (Landes, 1946) the endosperm is uniformly filled with storage food material except one layer lying in close contact with the persistent nucellus at the chalazal end. However, in *Chrozophora obliqua* (Kapil, 1956b; Fig. 2I), *Euphorbia hypericifolia* (Kapil, 1958) and *E. splendens* (Bhatnagar, 1970) the cells of the epidermal layer are devoid of any food reserves.

CULTURE EXPERIMENTS

Several attempts have been made to grow immature and mature euphorbiaceous endosperm in vitro with a view to (a) ascertaining its growth requirements, (b) inducing organ formation, (c) studying the metabolic pathways operating in the tissue at various stages of development and (d) understanding the relationship between the endosperm and embryo.

As early as 1944 LaRue observed one instance of roots developing from the endosperm of *Ricinus communis*. In subsequent experiments the mature endosperm of *Ricinus communis* (Mohan Ram and Satsangi, 1963; Srivastava, 1971a), *Putranjiva roxburghii*, *Jatropha panduraefolia* (Johri and Bhojwani, 1965) and *Croton bonplandianum* (Bhojwani, 1966) yielded a continuously growing undifferentiated tissue when grown along with the embryo. Differentiation of cells with reticulate thickenings and formation of embryoids were noticed in the cultures of the mature endosperm of *Ricinus communis* (Satsangi and Mohan Ram, 1965). Their experiments show that by application of 2, 4-D in nutritive medium, the growth of the endosperm can be stimulated and that of the embryo inhibited. Continuous growth of the endosperm callus, however, requires association of kinetin and is further improved in subcultures by the addition of yeast extract. Continuous light was found to be favourable and total darkness inhibitory to growth.

In *Croton bonplandianum* also thick-walled cells differentiate frequently in the endosperm callus and embryo-like structures develop (Fig. 3A) up to the globular stage (Bhojwani, 1966). The endosperm remains unorganized on WM + 2,4-D + kinetin + yeast extract. However, if the callus is subcultured on White's medium alone or in combination with casein hydrolysate, several roots differentiate (Bhojwani and Johri, 1971; Fig. 3B). These are triploid and show a distinct root cap (Fig. 3C). Low auxin concentrations were found to enhance root formation, though the endogenous auxins are not essential for this.

In the studies mentioned above (Satsangi and Mohan Ram, 1965; Johri and

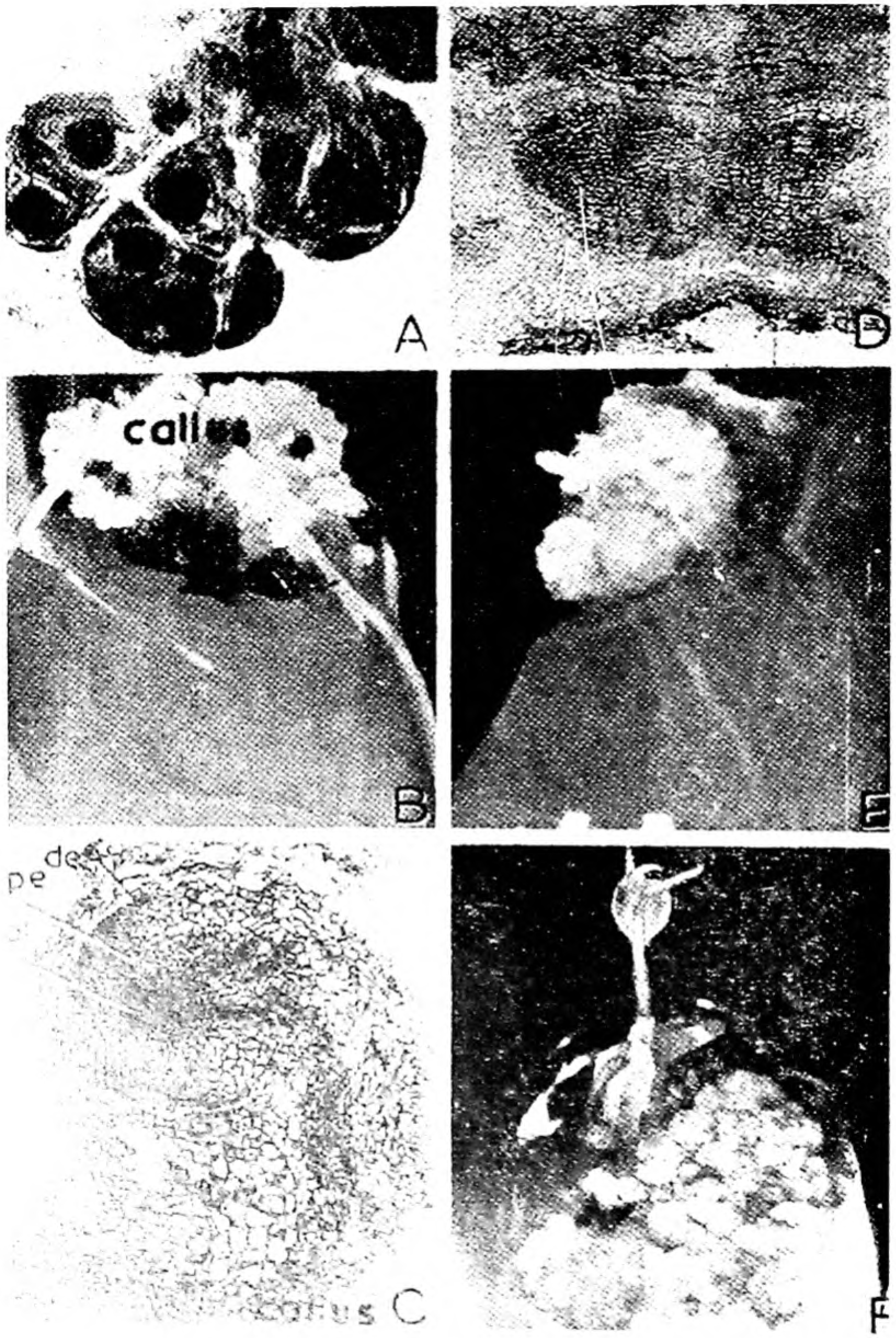


FIG. 3. A–C. *Croton bonplandianum*. A. Squash preparation from endosperm callus; cell aggregates resembling embryogenic stages. B. Six-week-old subcultures showing development of roots from callus. C. L.s. of an organized root primordium obtained in vitro. Root cap is clearly seen. D. *Ricinus communis*. Section of callusing endosperm showing cells simulating vascular bundles. E. *Jatropha panduraefolia*. Differentiation of roots and shoot bud from endosperm callus. F. *Putranjiva roxburghii*. Plantlet developing in 20-week-old cultures. (A, after Bhojwani, 1966; B, C, after Bhojwani and Johri, 1971; D, after Srivastava, 1971a; E, F, after Srivastava, 1971c).

Bhojwani, 1965; Bhojwani, 1966; Srivastava, 1971a) the mature endosperm was found to proliferate only when it was planted with intact embryo. Bhojwani (1966), therefore, concluded that "activation of mature endosperm depends on some metabolites produced by the embryo". In *Croton bonplandianum* the embryo factor responsible for inducing growth of endosperm could be successfully replaced by presoaking pieces of endosperm in aqueous solution of gibberllic acid (Bhojwani, unpublished). More recently, Brown et al. (1970) succeeded in establishing callus growth from endosperm explants (at all stages of development) without embryo. However, they observed that the metabolic pathways which characterize the endosperm in vivo are not retained under the culture conditions. There is a marked reduction in the capacity for acetate metabolism inlong term cultures, the fatty acid synthesizing capability does not persist even for a short time and the process of lipid accumulation found in normal developing endosperm is replaced in culture by a process which consumes the already accumulated oil reserves. According to these authors "it is quite possible that the manipulation of the components of the culture medium may affect the matabolism of the tissue".

Recently, Srivastava (1971a) observed differentiation of a peripheral zone of cambium-like cells followed by thick-walled cells simulating vascular bundles in the callusing mature endosperm of *Ricinus communis* (Fig. 3D) but was unable to induce organogenesis in callused endosperm. In contrast, in *Jatropha panduraefolia* (Srivastava, 1971b) differentiation of both roots and shoots occurred (Fig. 3E). White's medium supplemented with 2,4-D, kinetin and yeast extract proved best for proliferation of the endosperm (with embryo). On transference to WM+kinetin+casein hydrolysate+NAA, both shoot buds and roots differentiated on the callus. The roots grew normally whereas the shoot buds failed to develop leaves. However, in *Putranjiva roxburghii* differentiation of complete plantlets was achieved (Srivastava, 1971c; Fig. 3F).

Although, it has been possible to obtain a continuously growing tissue from the endosperm of several plants when cultured along with the embryo, organogenesis has been recorded only in a few cases. In fact, to date *Ricinus communis, Croton bonplandianum, Jatropha panduraefolia* and *Putranjiva roxburghii* constitute perhaps the only instances among autotrophic plants where triploid endosperm has been successfully induced to yield organogenesis.

BIOCHEMICAL AND PHYSIOLOGICAL STUDIES

These are mostly confined to the seeds of *Ricinus communis* which contain, on a dry weight basis, nearly 55% oil and 20% proteins (Canvin, 1963). Oil synthesis starts 6-9 days after pollination, is at its maximum during 20 to 40-day period after pollination, and terminates 60 days after pollination, upon maturation and dehydration of the seed. The percentage composition of the oil varies according to the age of the seed. Ricinoleic acid, the major component of castor oil is not present in the initial stages when the oil consists chiefly of palmitic acid, linoleic acid, oleic acid and linolenic acid. Synthesis of ricinoleic acid is very rapid 15 days after pollination. Canvin (1965a) showed that under aerobic conditions oleic acid was first to be formed in castor bean endosperm and was the precursor of ricinoleic acid. Linoleic acid is an intermediate stage in the conversion of oleic acid into ricinoleic acid. On the other hand, in the absence of oxygen stearic acid and palmitic acid are formed. That the oleic acid is a precursor of ricinoleic acid in castor endosperm is confirmed by Galliard and Stumpf (1966). The oil content and the composition of the castor oil is not affected by the changes in temperature (Canvin, 1965b). The co-factors required for oleic acid synthesis, with acetyl Co-A as substrate were: ATP, Mn, HCO_3, NADPH and NADH. In the presence of NADH alone stearic acid was the main product (Drennan and Canvin, 1969). In developing endosperm all the enzymes required for oleic acid synthesis have been found to be associated with subcellular particles, which are either proplastids or some other new unique particles (Zilkey and Canvin, 1969).

In contrast to oil, protein is synthesized during the entire period of seed development, but the rate of synthesis is more rapid in the first few days of endosperm formation (Canvin, 1963). There seems to be an inverse relation between the oil and protein content but the factors responsible for diversion of acetate to lipids or proteins remain to be studied (Canvin, 1965b).

At the time of maturation all metabolic activities in the endosperm are at a minimum. Alberghiana (1964) showed that the rate of oxygen uptake which was maximum during the period of optimum oil synthesis decreased to zero in the mature seed. After germination the metabolism in the endosperm is mainly directed to rapid catabolism of oil reserves to sucrose (Beevers, 1961).

Many attempts have been made to elucidate clearly the mechanism of lipid catabolism in castor bean endosperm. The fatty acids are broken down to acetyl units via B-oxidation (Yamada and Stumpf, 1965). Kornberg and Beevers (1957) had earlier suggested that the glyoxylate cycle, coupled with a reversal of glycolysis, afforded a plausible route for conversion of acetate to sugar. Canvin and Beevers (1961) also demonstarated that the major fate of acetate is conversion to sucrose by way of dicarboxylic acids produced in glyoxylate cycle. These authors believe that tricarboxylic acid cycle (TCA) is not operative in the endosperm of a germinating castor bean. In *Ricinus communis* the glyoxylate cycle has been shown to be located in unique subcellular particles called glyoxysomes (Breidenbach and Beevers, 1967). Cooper (1971) has suggested that in *Ricinus* endosperm, besides glyoxysomes, the endoplasmic reticulum may also be the site of activation of long chain fatty acids. According to Cooper and Beevers (1969) the generation of acetyl Co-A from long chain fatty acids in glyoxysomes explains why acetate escapes oxidation through TCA cycle and is exclusively catabolised through the glyoxylate cycle. However, in a recent study Brown et al. (1970) observed that in in vitro studies, in 4-day germinated endosperm tissue the metabolism of acetate was consistent with the glyoxylate cycle whereas in 2-day germinated endosperm it occurred mainly via the TCA cycle with possibly some participation of glyoxylate cycle.

Brown et al. (1970) found that in the developing endosperm, from 24-36 days after fertilization, acetate metabolism was brought about by TCA cycle. More recently Agarwal and Canvin (1971a) have shown that pentose phosphate pathway (PPP) is also operative in the *Ricinus* endoperm at all stages of seed development. Depending upon the age of the seed, 5-12% of the glucose used was metabolized via the PPP and 88-95% via the TCA cycle (Agarwal and Canvin, 1971b). They suggested a general correlation between the rates of lipid synthesis and the contribution of PPP in glucose dissimilation, although the NADPH produced in PPP is not the only source of reducing hydrogen required in lipid synthesis. This is in agreement with the views of Cornaggia (1964) who showed an increase in the activity of glucose-6-phosphate dehydrogenase when castor bean endosperm was storing fat.

Discussion and Conclusions

The primary endosperm nucleus in the Euphorbiaceae is diploid to 15-ploid. However, this knowledge is based entirely on the number of polar nuclei taking part in the organization of the fusion nucleus rather than on cytological studies. For authentic information it is desirable to investigate the chromosome numbers, especially in plants in which the type of embryo sac development itself is disputed.

Whatever the constitution of the primary endosperm nucleus, the development follows almost a similar course in all the species investigated, and is invariably of the Nuclear type. This is in contrast to the ontogeny of embryo sac and embryo which exhibit a great diversity. There is an instance of only one genus, *Croton*, where the endosperm develops a chalazal haustorial structure. In a couple of other examples, i.e., *Euphorbia dulcis* and *E. helioscopia*, enucleate cytoplasmic vesicles have been observed at the free nuclear stage of endosperm. According to D. Singh (1964) the likely role of such nodules in Cucurbitaceae is to fill up the large central vacuole in the endosperm. He also states that these nodules are generally better developed in plants of higher altitude or temperate regions. Incidentally, the materials of both *E. dulcis* (Kapil, 1961) and *E. helioscopia* (R.P. Singh, 1969) were also collected from such climatic regions.

Another aspect of study which has hardly been commented upon by the investigators, is the characteristic synchrony in the earlier nuclear divisions of endosperm which is met with in several taxa of the Euphorbiaceae. Some authors have speculated about the existence of substances in the endosperm which are responsible for synchrony in division. Tischler (1922, quoted from Erickson, 1964) believed in the presence of a diffusible 'Reizostoffe' in the embryo sac. Dixon (1946), on the basis of his study on *Fritillaria imperialis*, suggested that there may be a mitotic hormone in the endosperm. He observed transverse bands of nuclei which were in successively more advanced stages of mitosis from the antipodal to the micropylar end of the ovule and concluded that a hormone which initiates mitosis diffused from the micropylar to the chalazal end. At later stages, when cell walls appear, the synchrony is not so apparent, because the free diffusion of the hormone which was possible in free nuclear state is hindered by the walls. This hypothesis does not seem to hold good for *Chrozophora obliqua* (Kapil, 1956b) and *Securinega leucopyra* (Bhatnagar, unpublished) where sometimes in the same embryo sac the nuclei in the micropylar and chalazal regions were in resting condition but those in the middle part were in metaphase or late telophase. Similarly, in *Euphorbia geniculata* (Bhatnagar, 1970), the nuclei in the micropylar and chalazal regions have been observed to be in metaphase and those in the middle in anaphase. A condition, exactly the reverse of what has been cited by Dixon, exists in *Ceratocephalus falcatus,* a member of the Ranunculaceae (Bhandari and Asnani, 1968). In this plant the division figures are at a successively advanced stages of mitosis from the micropylar to the chalazal end. These examples, thus, do not support Dixon's hypothesis.

Zeuthen (1964) believes that continuous synchrony is monitored by continued changes in the environment, and constant environment brings about desynchronizing tendencies. Such a possibility cannot be ruled out for the endosperm in angiosperms. As compared to gymnosperms, the embryo sac in flowering plants is covered not only with several layers of nucellus and integuments of the ovule but also by the ovary wall and is thereby better protected from environmental changes, thus leading to a much lower degree of synchrony in the development of endosperm in angiosperms. However, this observation needs confirmation from more extensive data. It would also be interesting to know if the young endosperm with synchronously dividing nuclei, continues to exhibit synchrony or not when grown in vitro under changing environmental conditions.

Experiments with the anthers of *Lilium longiflorum* (Taylor and McMaster, 1954; Foster and Stern, 1959; Hotta and Stern, 1961; Takats, 1962) and *Trillium erectum* (Stern and Kirk, 1948; Stern, 1956, 1958), in which both the meiosis of microsporocytes and mitosis of microspores are synchronized, have shown striking correlation of the respiratory metabolism, radiation sensitivity and nucleic acid synthesis with the cytological events and have thrown some light on the physiology of cell division. The endosperm would perhaps fare better for similar investigations. Besides, it would provide comparative data with purely paternal tissue. In fact, the synchronously dividing nuclear endosperm would prove more suitable since (i) it lacks cell walls, (ii) it is relatively free of cytoplasmic inclusions and is devoid of stored food in early stages, (iii) it has a large absorbing surface, (iv) it provides greater manoeuvrability for experimental or environmental studies and (v) it can be easily dissected out in part or in full.

The participation of the male gamete in the organization of the primary endosperm nucleus constitutes a regular and important event in the life of a flowering plant and has been commented upon by various authors. Nawaschin (1898) and Sargant (1900) considered triple fusion as "an act of true fertilization" involving one male gamete and one normal female element (upper polar nucleus) and compared it with the sexual fusion of male gamete with the egg. According to them the participation of the chalazal polar nucleus in this process (triple fusion) disturbs the balance and brings about the degeneracy of the resulting tissue, i.e., the endosperm. On the other hand, Strasburger (1900) was of the opinion that the involvement of the male gamete in triple fusion served only as a growth stimulus. Sargant (1900) also suggested that the purpose of triple fusion could be to maime the "second embryo" (endosperm) from the beginning so that the first embryo (zygotic) survived without struggle and the 'maimed embryo' played a nutritional role instead. Culture experiments have shown that conditions favourable for proliferation of the endosperm are antagonistic to the growth of the embryo and vice versa, emphasizing thereby their equal efficacy. That the endosperm is potentially a second embryo, is further substantiated by experimental studies on *Croton bonplandianum* (Bhojwani and Johri, 1971), *Jatropha panduraefolia* and *Putranjiva roxuburghii* (Srivastava, 1971b, c). In these plants organogenesis has been obtained from the triploid endosperm with the help of growth regulators. As far as the instances of autonomous growth of the endosperm are concerned, they do not appear to be different from those of the parthenogenetic development of the embryo.

If the embryo and endosperm have similar attributes, what then is the cause of their differential behaviour? An attempt to answer this question was made as early as 1900 by Sargant. She believed that the endosperm was 'maimed' due to the imbalance caused by the participation of the chalazal polar nucleus in triple fusion. This view becomes unacceptable in the light of subsequent discoveries of other types of embryo sacs, particularly the Oenothera type, as well as the reduced embryo sacs, like that of *Butomopsis* (Johri, 1936) which have only one polar nucleus, and of some members of Balanophoraceae (Fagerlind, 1945a, b) in which the upper polar nucleus alone is fertilized. In Euphorbiaceae it is clearly seen that the number of nuclei involved in secondary fusion (2-15), sometimes even coming from all the four poles of the embryo sac, does not make much difference to the development and fate of the endosperm. The zygotic and the primary endosperm nuclei are placed not only in morphologically but probably also in physiologically different cytoplasmic surroundings which seem to play a decisive role in their differential behaviour. Qualitative and quantitative histochemical studies of these nuclei and their environments, particularly in respect of nucleic acids, may perhaps elucidate the course of their development.

Commenting upon the nature of endosperm, Thomas (quoted in Maheshwari, 1950) and Némec (1910) stated that the purpose of the "hybridity" of this tissue could be to provide the embryo with a physiologically compatible nutritive tissue (i.e. of a similar mixed stock), instead of a purely maternal tissue. The association of nucellar embryos with autonomous endosperm in *Alchornea ilicifolia* (Strasburger, 1878), *Euphorbia dulcis* and *Mallotus japonicus* indirectly lends credence to this view. The hybrid nature of the endosperm may also be providing it an edge over the surrounding tissue (i.e. the nucellus) of the ovule for deriving its nutrition (Brink and Cooper, 1940, 1947), for it is seen that in *E. dulcis* and *M. japonicus* the autonomously developed endosperm is much less aggressive, and instead of destroying the surrounding nucellar cells, permits their proliferation into embryos. It remains to be judged whether this interpretation holds good for other plants as well.

In 1938, V.S. Rao suggested a correlation between the occurrence of Nuclear endosperm and embryo differentiation. According to him the easy availability of nourishment from free nuclear endosperm helps in early differentiation of the embryo. This cannot be confirmed on the basis of our observations in the Euphorbiaceae wherein wall formation, which mostly begins first in the region around the embryo, is usually initiated when the proembryo is at early globular stage, and, by the time it is at the late globular or early heart-shaped stage, the endosperm is completely cellular. Similar situations occur in many other families such as Garryaceae, Hamamelidaceae, Proteaceae, Ranunculaceae, Umbelliferae etc. In view of divergent data, more statistical information needs to be collected before a true correlation between the type of endosperm and differentiation of embryo can be established.

The exact role of endosperm in the organization of the embryo requires further investigation. The differentiation of the zygotic embryo is often slow or lacking in plants which depend for their nutrition on sources other than the endosperm as in Orchidaceae. In such examples the developing embryo has to depend on either the nucellus, integuments or chalaza. Although there are exceptions in which the differentiation of the embryo is normal inspite of almost complete absence of the endosperm (e.g., Podostemaceae) but in these cases the nutrition is derived through special structures such as pseudo-embryo sac besides the suspensor haustoria.

It appears that there is some sort of reciprocally beneficial relationship between the endosperm and the embryo as is also indicated by culture experiments. When the young proembryo is dissected out and cultured without endosperm its growth and differentiation usually differs from growth in vivo (see Sanders and Ziebur, 1963). Similarly when the endosperm is cultured without embryo there is a decrease in its biochemical capabilities (Brown et al., 1970). However, it remains to be confirmed that these differences in the growth of the embryo and the endosperm in vivo and in vitro are not due to the manipulation of physical or chemical factors under culture conditions but due to the mutual relationship between the embryo and the endosperm.

Mention may also be made here of the relationship of the endosperm with its surrounding nucellar tissue. Muzik (1954) believes that in *Hevea brasiliensis*, "during growth the endosperm was absorbed by the nucellus". This is extremely unlikely. In our opinion, as is usual, it is the endosperm which obtains nutriment from the nucellus. According to Nakajima (1962) the natural growth of the endosperm in cucumber (Cucurbitaceae) depends on auxins, cell division factors and organic substances which it does not produce itself but receives from the nucellus. Recently Gori et al. (1971) have shown the existence of spherical organelles produced from rough endoplasmic reticulum in the nucellar cells of many species of *Euphorbia*. They state that it is unlikely that the contents of these organelles are utilized by the nucellar cells themselves and that it is more probable that the material of organelles is transferred to the endosperm. Similar type of organelles, e.g., glyoxysomes (Breidenback and Beevers, 1967; Vigil, 1970) and the new unique subcellular particles which are the seat of oleic acid synthesis (Zilkey and Canvin, 1969) occur in the endosperm of *Ricinus communis* and these play a very active role in fat metabolism. It would be of relevance to ascertain the degree of similarity between the organelles found in nucellus of *Euphorbia* and the endosperm of *Ricinus communis*. Besides, it is important to determine if these or some other types of cytoplasmic inclusions, as yet unknown, could be responsible for the synthesis and storage of food in endosperm.

REFERENCES

AGARWAL, P. K. AND D. T. CANVIN. 1971a. Respiration of developing castor bean seeds. *Can. J. Bot.* **49**: 263-266.

——, AND ——. 1971b. Contribution of pentose phosphate pathway in developing castor bean endosperm. *Can. J. Bot.* **49**: 267-272.

ALBERGHIANA, F. A. M. 1964. Evoluzione del metabolismo ossidativo dei semi di Ricino durante la maturazione. *G. Bot. Ital.* **71:** 392-398.

BANERJI, I. 1949. A contribution to the life history of *Acalypha fallax* Muell. Arg. *Bull. bot. Soc. Beng.* **3:** 29-32.

BEEVERS, H. 1961. Metabolic production of sucrose from fat. *Nature, Lond.* **191:** 433-436.

BHANDARI, N. N., AND SHANTI ASNANI. 1968. Studies in the family Ranunculaceae. XI. Morphology and embryology of *Ceratocephalus falcatus* Pers. *Beitr. Biol. Pfl.* **45:** 271-290.

BHATNAGAR, A. K. 1970. *Embryological Studies on Euphorbia geniculata* Orteg. *and E. splendens* Boj. M.Sc. (Hons. School) thesis, Panjab Univ.

BHOJWANI, S. S. 1966. Morphogenetic behaviour of mature endosperm of *Croton bonplandianum* Baill. in culture. *Phytomorphology* **16:** 349-353.

——, AND B. M. JOHRI. 1971. Morphogenetic studies on cultured mature endosperm of *Croton bonplandianum*. *New Phytol.* **70:** 761-766.

BRINK, R. A., AND D. C. COOPER. 1940. Double fertilization and development of seed in angiosperms. *Bot. Gaz.* **102:** 1-25.

——, AND ——. 1947. The endosperm in seed development. *Bot. Rev.* **8:** 423-477.

BREIDENBACH, R. W., AND H. BEEVERS. 1967. Association of glyoxylate cycle enzymes in a novel sub-cellular particle from castor bean endosperm. *Biochem. Biophys. Res. Commun.* **27:** 462-469.

BROWN, D. J., D. T. CANVIN, AND B. F. ZILKEY. 1970. Growth and metabolism of *Ricinus communis* endosperm in tissue culture. *Can. J. Bot.* **48:** 2323-2331.

CANVIN, D. T. 1963. Formation of oil in the seed of *Ricinus communis* L. *Can. J. Biochem. Physiol.* **41:** 1880-1885.

——. 1965a The biosynthesis of long chain fatty acids in the developing castor bean. *Can. J. Bot.* **43:** 49-70.

——. 1965b. Incorporation of acetate-1-C^{14} into the long chain fatty acids of some oil seed crops. *Can. J. Bot.* **43:** 71-74.

——, AND H. BEEVERS. 1961. Sucrose synthesis from acetate in germinating castor bean. Kinetics and pathway. *J. Biol. Chem.* **236:** 988-995.

CARANO, E. 1926. Ulteriori osservazioni su *Euphorbia dulcis* L., in rapporto col suo comportamento apomittico. *Annali Bot.* **17:** 50-79.

COOPER, T. G. 1971. The activation of fatty acids in castor bean endosperm. *J. Biol. Chem.* **246:** 3451-3455.

——, AND H. BEEVERS. 1969. B-oxidation in glyoxysomes from castor bean endosperm. *J. Biol. Chem.* **244:** 3514-3520.

CORNAGGIA, M. P. 1964. Aspetti enzimatici della maturazionee germinazione dei semi di Ricino. *G. Bot. Ital.* **71:** 503-508.

DAVIS, G. L. 1966. *Systematic Embrynlogy of the Angiosperms.* John Wiley and Sons Inc., New York.

DESSIATOFF, N. 1911. Zur Entwicklung des Embryosackes von *Euphorbia virgata*. *Ber. dt. bot. Ges.* **29:** 33-39.

DIXON, H. H. 1946. Evidence for a mitotic hormone: observations on mitosis of the embryo sac of *Fritillaria imperialis*. *Scient. Proc. R. Dubl. Soc.* **24:** 119-124.

DONATI, G. 1912. Di alcune particolarita embriologiche in *Poinsettia pulcherrima*. *R.Gr. C.r. Acad. Lincei, Roma.* **21:** 512-514.

DRENNAN, C. H., AND D. T. CANVIN. 1969. Oleic acid synthesis by a particulate preparation from developing castor oil seeds. *Biochim. Biophys. Acta.* **187:** 193-200.

ERICKSON, R. O. 1964. Synchronous cell and nuclear division in tissues of the higher plants. In *Synchrony in Cell Division and Growth*. E. Zeuthen (ed.). New York.

FAGERLIND, F. 1945a. Bauder floralen organe bei der Gattung *Langsdorffia*. *Svensk bot. Tidskr.* **39:** 197-210.

——. 1945b. Bildung und Entwicklung des Embryosacks bei sexuallen und agamospermischen *Balanophora* Arten. *Svensk bot. Tidskr.* **39:** 65-82.

FOSTER, T. S., AND H. STERN. 1959. The accumulation of soluble deoxyribosidic compounds in relation to nuclear division in anthers of *Lilium longiflorum*. *J. Biophys. Biochem. Cytol.* **5:** 173-192.

GALLIARD, T., AND P. K. STUMPF. 1966. Fat metabolism in higher plants. XXX. Enzymatic synthesis of recinoleic acid by microsomal preparation from developing *Ricinus communis* seeds. *J. Biol. Chem.* **241:** 5806-5812.

GORI, P., G. SARFATTI, AND M. CRESTI. 1971. Development of spherical organelles from the endoplasmic reticulum in the nucellus of some *Euphorbia* species. *Planta* **29:** 133-143.

HOTTA, Y., AND H. STERN. 1961. Deamination of deoxycytidine and 5-methyldeoxycytidine in developing anthers of *Lilium longiflorum* (var. *craft*) *J. Biochem. Biophys. Cytol.* **9:** 279-284.

JOHRI, B. M. 1936. The life history of *Butomopsis lanceolata* Kunth. *Proc. Indian Acad. Sci. Sect.* B. **4:** 139-162.

——, AND S. S. BHOJWANI. 1965. Growth response of mature endosperm in cultures. *Nature, Lond.* **208:** 1345-1347.

——, AND R. N. KAPIL. 1953. Contribution to the morphology and life history of *Acalypha indica* L. *Phytomorphology* **3:** 137-151.

KAJALE, L. B. 1954. Fertilization and the development of embryo and seed in *Euphorbia hirta* Linn. *Proc. natn. Inst. Sci. India* B. **20:** 353-360.

——, AND K. S. N. MURTHY. 1954. The embryo sac of *Acalypha ciliata* Forsk. *J. Indian bot. Soc.* **33:** 417-422.

KAPIL, R.N. 1956a. Development of embryo sac and endosperm in *Chrozophora rottleri* A. Juss.—A reinvestigation. *Bot. Gaz.* **117:** 243-247.

——. 1956b. A further contribution to the morphology and life history of *Chrozophora*

Neck. *Phytomorphology* **6:** 278-288.

——. 1958. *Morphological and Embryological Studies in Some Euphorbiaceae together with a Discussion on the Systematic position of the family.* Ph.D. thesis, Delhi Univ.

——. 1960. Embryology of *Acalypha* Linn. *Phytomorphology* **10:** 174-184.

——. 1961. Some embryological aspects of *Euphorbia dulcis* L. *Phytomorphology* **11:** 24-36.

Kornberg, H. L., and H. Beevers. 1957. The glyoxylate cycle as a stage in the conversion of fat to carbohydrate in castor beans. *Biochim. Biophys. Acta.* **26:** 531-537.

Landes, M. 1946. Seed development in *Acalypha rhomboidea* and some other Euphorbiaceae. *Am. J. Bot.* **33:** 562-568.

Larue, C. D. 1944. Regeneration of endosperm of gymnosperms and angiosperms. *Am. J. Bot.* **31:** 45.

Lyon, F. M. 1898. A contribution to the life history of *Euphorbia corollata. Bot. Gaz.* **25:** 418-426.

McCann, L. P. 1945 Embryology of the tung tree. *J. agric. Res.* **71:** 215-229.

Maheshwari, P. 1950. *An Introduction to the Embryology of Angiosprrms.* McGraw Hill Book Co. Inc., New York.

——, and B. M. Johri. 1941. The embryo sac of *Acalypha indica* L. *Beih. bot. Zbl.* **61A:** 125-136.

*Modilewski, J. 1909. Zur Embryobildung von *Euphorbia procera. Ber, dt. bot. Ges.* **27:** 21-26.

——. 1911. Über die abnormale Embryosackbildung bei *Euphorbia palustris* L. und anderen Euphorbiaceen. *Ber. dt. bot. Ges.* **29:** 430-436.

Mohan Ram, H. Y., and Asha Satsangi. 1963. Induction of cell division in mature endosperm of *Ricinus communis* during germination. *Curr. Sci.* **32:** 28-29.

Mukherjee, P. K. 1957. Studies on the embryology of *Euphorbia hypericifolia* Linn. *Bull. bot. Soc. Univ. Saugar.* **9:** 7-18.

——. 1958. The female gametophyte of *Acalypha malabarica* Muell. with a brief discussion on the Penaea type of embryo sac. *J. Indian bot. Soc.* **37:** 504-508.

——. 1964. Further contribution to the embryology of genus *Acalypha* Linn. *Proc. natn. Acad. Sci. India.* **B 35:** 327-337.

——, and M. D. Padhye. 1964. Contribution to the embryology of the genus *Phyllanthus* Linn. *Proc. natn. Acad. Sci. India.* **B. 34** 117-128.

Muzik, T. J. 1954. Development of fruit, seed, embryo, and seedling of *Hevea brasiliensis. Am. J. Bot.* **41:** 39-43.

Nakajima, T. 1962. Physiological studies of seed development, especially embryonic development and endeosperm development. *Bull. Univ. Osaka Prefact.* B. **13:** 13-48.

*Nawaschin, S.G. 1898. Resultate einer Revision der Befruchtungsvorgänge bei *Lilium martagon* und *Fritillaria tenella. Izv. imp. Acad. Nauk.* **9:** 377-382.

Némac, B. 1910. *Das Problem der Befruchtungsvorgänge und andere Zytologische Fragen.* Berlin.

Pal, Aruna. 1972. Structure and development of seeds in *Croton oblongifolius* Roxb. *Proc. 59th Indian Sci. Congr. Calcutta.* Pt. III Abstr. 330.

Raju, M. V. S., and A. Nagaraja Rao. 1953. The development of the male and female gametophytes in *Mallotus albus* Muell. *J. Mysore Univ.* **13:** 5-8.

Rao, A. N. 1964. Notes on embryology of *Hevea brasiliensis. Curr. Sci.* **33:** 739-740.

Rao, V. S. 1938. The correlation between embryo type and endosperm type. *Ann. bot.* **2:** 535-536.

*Sargant, Ethel. 1900. Recent work on results of fertilization in angiosperms. *Ann. Bot.* **14:** 689-712.

Sanders, M. E., and N. K. Ziebur. 1963. Artificial culture of embryos. In *Recent Advances in the Embryology of Angiosperms.* Maheshwari, P. (ed.). Intern. Soc. Pl. morph., Delhi.

Satsangi, Asha, and H. Y. Mohan Ram. 1965. A continuously growing tissue culture from the mature endosperm of *Ricinus communis* L. *Phytomorphology* **15:** 26-30.

Schürhoff, P. N. 1926. *Die Zytologie der Blütenpflanzen.* Stuttgart.

Sharma, K. D. 1955. Cyto-embryological studies in some Indian Euphorbiaceae. *Sci. Cult.* **21:** 270-271.

Singh, D. 1964. Cytoplasmic nodules in the endosperm of angiosperms. *Bull. Torrey bot. Club.* **91:** 86-94.

Singh, R. P. 1954. Structure and development of seeds in Euphorbiaceae: *Ricinus communis* L. *Phytomorphology* **4:** 118-123.

——. 1965. Structure and development of seeds in *Codiaeum variegatum. J. Indian. bot. Soc.* **44:** 205-210.

——. 1969. Structure and development of seeds in *Euphorbia helioscopia* Linn. *Bot. Mag., Tokyo.* **82:** 287-293.

——, and Suman Chopra. 1970. Structure and development of seeds in *Croton bonplandianum. Phytomorphology* **20:** 83-87.

Srivastava, P.S. 1971a. In vitro growth requirements of mature endosperm of *Ricinus communis. Curr. Sci.* **49:** 337-339.

——. 1971b. In vitro induction of triploid roots and shoots from mature endosperm of *Jatropha panduraefolia. Z. Pflanzenphysiol.* **66:** 93-96.

——. 1971c. *Morphogenic Studies on Mature Endosperm of Jatropha, Putranjiva* and *Ricinus.* Ph. D. thesis, Delhi Univ.

Srivastava, R. K., and G. P. Agarwal. 1953. Development of female gametophyte and endosperm in *Chrozophora rottleri. Bot. Gaz.* **114:** 348-350.

Stern, H. 1956. Sulfhydryl groups and cell division. *Science Wash.* **124:** 1292-1293.

——. 1958. Variation in sulfhydryl concentration during microsporocyte meiosis in anthers of *Lilium* and *Trillium. J. Biophys.*

Biochem. Cytol. **4:** 157-161.

——, AND P. L. KIRK, 1948. The oxygen consumption of the microspores of *Trillium* in relation to the mitotic cycle. *J. Gen. Physiol.* **31:** 243-248.

STRASSBURGER, E. 1878. Über Polyembryonie. *Jena. Z. Naturw.* **12:** 647-670.

——. 1900. Einige Bemerkungen zur Frage nach der doppelten Befruchtung bei den Angiospermen. *Bot. Ztg.* **58:** 293-316.

SWAMY, B. G. L., AND B. P. BALAKRISHNA. 1946. Female gametophyte of *Acalypha tricolor*. *J. Indian bot. Soc.* **25:** 67-69.

TAKATS, T. 1962. An attempt to detect utilization of DNA breakdown products from the tapetum for DNA synthesis in the microspores of *Lilium longiflorum*. *Am. J. Bot.* **49:** 748-758.

TATEISHI, S. 1927. On the development of the embryo sac and fertilization of *Acalypha australis* L. *Bot. Mag. Tokyo.* **31:** 477-485.

TAYLOR, J. H., AND R. D. MCMASTER, 1954. Autoradiographic and microphotometric studies of deoxyribose nucleic acid during microgametogenesis in *Lilium longiflorum*. *Chromosoma.* **6:** 489-521.

THATHACHAR, T. 1952. Morphological studies in the Euphorbiaceae: 1- *Acalypha lanceolata* Willd. *Phytomorphology* **2:** 197-201.

——. 1953a. Morphological studies in the Euphorbiaceae. *J. Mysore Univ.* **13:** 43-68.

——. 1953b. Morphological studies in Euphorbiaceae. II. *Mallotus philippensis* M. Arg. *Proc. natn. Inst. Sci. India* **B19:** 469-474.

VENKATESWARLU, J., AND P. N. RAO. 1963. Endosperm in Euphorbiaceae and occurrence of endosperm haustoria in two sps. of *Croton* Linn. *Curr. Sci.* **32:** 514-516.

VENTURA, M. 1934. Sulla poliembrionia di *Mallotus japonicus* Muell. Arg. *Ann. Bot.* **20:** 568-578.

VIGIL., E. L. 1970. Cytochemical and developmental changes in microbodies (glyoxysomes) and related organelles of castor bean endosperm. *J. Cell. Biol.* **46:** 435-454.

WENIGER, W. 1917. Development of embryo sac and embryo in *Euphorbia preslii* and *E. splendens*. *Bot. Gaz.* **63:** 266-281.

YAMADA, M, AND P. K. STUMPF. 1965. Fat metabolism in higher plants. XXIV. A soluble B-oxidation system from germinating seeds of *Ricinus communis*. *Plant Physiol.* **40:** 653-658.

ZEUTHEN, E. 1964. *Synchrony in Cell Division and Growth*. John Wiley and Sons Inc., New York.

ZILKEY, B., AND D. T. CANVIN. 1969. Subcellular localization of oleic acid biosynthesis enzymes in developing castor bean endosperm. *Biochem. Biophys. Res. Commun.* **34:** 649-653.

---

* not seen in original.

Adv. Pl. Morph 1972: 394–407

SOME THOUGHTS OF A PHYLOGENETIC ANATOMIST ON THE EVOLUTION OF THE FLOWER. I.

BY M. F. MOSELEY

University of California, Santa Barbara, U.S.A.

ABSTRACT

The two principal arguments expressed and supported in this paper are as follows:

1. There are numerous examples in the literature on floral anatomy where interpretations of conservative vasculature seem to be accurate, plausible, logical and reasonably unassailable. There are, on the other hand, equally unassailable instances where changes in the floral vasculature have obviously kept pace with, or even preceded, other morphological and anatomical evolutionary changes. There is no rational reason at present to assume that floral vascular systems are either typically conservative or completely co-evolutionary with other adaptational changes in flowers and fruits.
2. The Magnoliales and Trochodendrales (sensu Cronquist, 1968), both ranalean, possess the most primitive levels of advancement of the greatest number of extrinsic morphological and anatomical evolutionary trends to be found in any of the various dicotyledons often suggested as "Primitive". The extrinsic trends utilized for comparison are those which were initiated, and their directions determined, in taxa other than, and antecedent to, the angiosperms. The trends used are: reduction in lengths of imperforate and of perforate tracheary elements, the change from sieve cells to sieve tubes, and the change from 1—sulcate pollen grains to other types. Added to these are two additional trends, also possibly extrinsic, the aquisition of perforation plates and the gradual transformation of the sieve-tube elements to more specialized forms.

Other matters discussed were:

1. a re-affirmation of the need for consideration of maximum kinds of characters in any phylogenetic study:
2. the opinon that the multiplicity of theories for the origin of the flower is due, in part, to a misunderstanding of basic concepts of vascular plant anatomy.

INTRODUCTION

In a recent paper, Carlquist (1969) scrutinized somewhat justifiably certain concepts held nearly inviolable by floral anatomists. I wish to write principally to the summary rejection of concepts and views, because I consider that the truths Carlquist and others seek lie between the old concepts and his principles. We should not exchange one set of rigid views for another. My comments are restricted chiefly to Carlquist's (1969) Principles 1, 2, 3, 5 and 6 wherein I claim some competence.

PLASTICITY OF ANGIOSPERMS

Carlquist (1969) has questioned the concept that the vascular systems of flowers are conservative in his Principles 1, 2, 3 and 5. Principle 1, however, contains a second idea interwoven with the problem of vascular tissue conservatism. His first principle follows: "1. Anatomy of flowers can be studied meaningfully only in relation to adaptations for particular modes of pollination, dispersal, and allied functions. Structures in flowers are, with very few exceptions not functionless relicts but positive adaptations. Angiosperms are an exceptionally plastic and rapidly-evolving group of plants, and may be expected to lose non-functional structures rapidly. Venation relates, with virtually no exceptions, to contemporary functions". I agree with the view that angiosperms are exceptionally plastic and rapidly evolving, but only partly with other aspects of this principle. I suggest that the first Principle be restricted to the widely acceptable thoughts regarding plasticity and structural-functional relations, and thereby permit consideration of the relatively controversial concept of the conservatism of the vascular system separately. Principle 1 might read: The anatomy of flowers is frequently closely related to adaptations for particular modes of pollination, dispersal mechanisms, and allied functions and attention should be directed, without fail, to these relationships in descriptive or phyletic studies. Since angiosperms are an exceptionally plastic and rapidly evolving group of plants, it is to be expected that they may lose non-functional structures rapidly, or modify them for other functions.

THE CONCEPT OF THE CONSERVATISM OF THE FLORAL VASCULAR SYSTEM

The remainder of Principle 1, and virtually all of Principles 2, 3 and 5 together are different aspects of one concept and are discussed herein as one problem. The viewpoint expressed by Carlquist (1969) is, in my opinion, extreme, dogmatic and not accurate.

Principles 2, 3 and 5 read, respectively: "2. Supposed conservatism of the vascular system of the flower is the insufficient and fallacious framework on which most phylogenetic interpretations of floral anatomy still rest. In fact, with certain exceptions. . . ., there is no reason for believing that the vascular system lags behind external form to any appreciable degree. 3. With very few exceptions, the majority of flowers can be expected to show evolution of vascular system at approximately the same rate as other anatomical and morphological features of the flower. 'Conservatism' can be expected where function and total amount of venation remain roughly the same during phylesis, but degree of union between adjacent bundles does change. Lagging in degree of union of bundles (really not conservatism at all, strictly speaking) is the only clear exception to evolution of vascular bundles contemporaneously with evolution of other floral features. . . . .5. Presence of vascular bundles should be interpreted as functional structures related to actual physiological activity, not as vestiges that do not function yet have not yet been lost. Vascular bundles differentiate according to principles of morphogenesis, and not in response to non-existent organs or to past events in the phylesis of a group. The efficiency of angiosperms is such that vascular bundles retained as in an historical archive are not to be expected."

To accept these statements as offered ignores a vast literature of floral anatomy replete with descriptions and diagrams of vein stubs, and stelar lacunae associated with reduced or apparently lost floral organs, and variously fused or amplified vascular systems lagging behind organ connation. These innumerable instances of conservatism do not vanish because of statements which imply their non-existence, although some of them, probably, could undoubtedly be better explained in other ways. I doubt that the plasticity of angiosperms is so great that the vascular systems and grosser morphology of flowers are as completely integrated and co-evolutionary as Carlquist (1969) implies. Vestigial floral organs have frequently become nectariferous glands, dispersal structures, etc., but it has often been stunted vascular supplies to them which have revealed their former morphologies.

At one exterme among floral anatomists, morphologists, and systematists, there are those who see relatively little or no phyletic value in floral vascular systems (e.g., Arber, 1933; Carlquist, 1969; Lam, 1952; Troll, 1933). From this nihilistic extreme, all gradations of reliance on vascular sytems exist with, perhaps, a large number of writers depending too greatly on its supposed slowly mutable nature (e.g., Douglas, 1957; Eames, 1961; Wilkinson, 1948). It is my opinon that either approach is unreliable, often partly emotional rather than objective, and hence ascientific. The only way to come to such positive conclusions as those reached by Carquist (1969) is to examine the numerous (innumerable, would be a better term) publications on floral anatomy, or re-examine the flowers concerned. I doubt that this has been done.

Risking the same criticism, I would like to mention briefly that there are, of course, many publications describing situations where the vascular systems of flowers are not conservative. Puri (1951) has mentioned many of these, and I shall not discuss them again (e.g., Arber, 1933; Blaser, 1941; Boothroyd, 1930; Joshi, 1936; Koch, 1930; Moore, 1936; Smith, 1926).

As I view the concept of the conservative nature of floral vasculature, there seem to be two kinds of evidences whereby one may discuss it and evaluate it. The first approach is to recall examples where it has been of striking value in solving structural peculiarities, adaptations, phylogenetic relationship, or adaptations satisfactorily. Obviously, only a few examples can be offered.

The nectaries of *Salix* (Fisher, 1928) are especially pertinent here. In some species, they receive no vascular supplies, indicating, perhaps, that vascular tissue (Fig. 1) is not necessary for their functioning. In other species, the nectaries receive varying numbers of traces from the full complement of three received by petals to one. In others, only parts of traces are present, apparently discontinuous with the source. In three species, there are no nectaries, but petals or intermediate structures (Fig. 2, 1) stand in place of them. This appears to be a reduction and transformation with the vascular bundles "lagging" behind the grosser evolutionary changes.

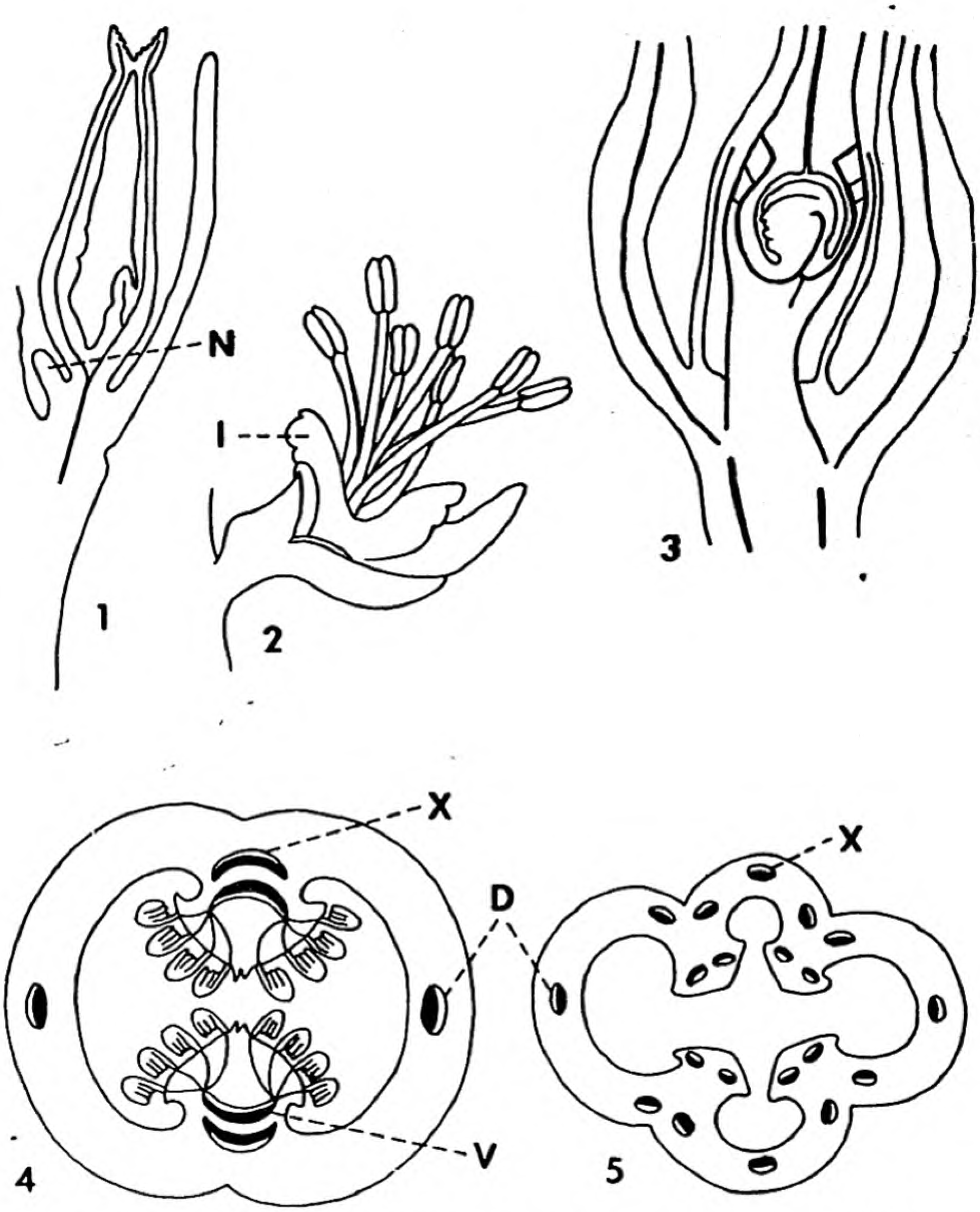
The extensive, comparative study of E. C. Abbe (1935) reveals many superb examples of the gradual reduction of floral parts, flowers, and inflorescences in Betulaceae. Morphological changes have been followed more slowly by reduction of vascular supplies. The illustrations are convincing. The gross condensation and compaction of the flowers are related, of course, to modification for wind pollination. There is certainly correlation of morphology and anatomy in this instance; it is hardly necessary to mention it.

In *Alnus lanata*, for example, diminutive traces are all that now represent two secondary bracts of a staminate cymule. In *Alnus rhombifolia*, the secondary axis of a cymule, which in *A. incana* consists of a basal bundle and four branches, is reduced to a trace. In *Ostrya*, secondary and tertiary bracts have fused, but the vascular supplies to the formerly separate organs are present.

Sastri (1959) has shown that the vascular supply to the sub-basal ovule in *Myristica fragrans* originates high in the walls of the ovary from both dorsal and ventral carpellary vascular supplies (Fig. 3). This would seem to be a good example of the retention of a more ancient vascular pattern in the genome of the organism.

A convincing example of a conservative vascular supply has been demonstrated by Puri (1950) in his paper in *Crataeva religiosa* Forst. Avoiding some of the details, the normal bicarpellary ovary seen in a transverse mid-section has two carpels lateral to the median-posterior axis with the locules confluent over part of the vertical distance of the ovary (Fig. 4). The dorsal vascular carpellary (Fig. 4, D) bundles are typical but the principal inverted ventral vascular supplies to adjacent carpels form an arc on either side of the locule in the anterio-posterior positions (Fig. 4, V). Branches from these bundles supply the ovules. Centrifugal to each arc of a ventral carpellary strand (Fig. 4, V) is another arc of normally oriented vascular tissue (Fig. 4, X) each occurringas a bundle or a group of bundles. Puri (1950) termed these the lateral vascular strands. These strands might be considered as having evolved to bring food to the wall of the ovary, or to have some function in dispersal, as indeed they may. They are, nevertheless, vestigial dorsal carpellary bundles. This can be seen very clearly in tricarpellary and tetracarpellary ovaries (Fig. 5) which occur in some flowers. In the latter, antero-posterior fertile locules occur, and the accessory, normally oriented strands become the dorsal carpellary bundles. The ventral carpellary bundles for the accessory carpels are derived from the ventral inverted supply already described.

The second approach to the concept of the conservatism of the floral vascular systems is rather different. Whereas the first approach has been to demonstrate changes in floral vascular systems which have often left vestigial vasculature and which have occurred as results of of morphological changes, probably in response to adaptation, I wish now to draw attention to certain basic, intrinsic characteristics of the floral vascular systems which are in themselves conservative, and subject to almost no change. It appears to me that these fundamental vascular characters, so constant and so widespread in flowers, changing only under very great adaptation, strongly substantiate the idea that floral vasculature is conservative. (1) I tend to think that the basic stem and appendage structure can be demonstrated to some extent in nearly any flower. There is a receptacular system, representing the stele of the floral axis composed of normally oriented vascular bundles, and traces from this, and/or an additional cortical system, to the floral parts. The receptacular and trace systems are often modified by strong condensation and connation, but the basic structure remains. (2) The three-veined venation of carpels with the veins in the characteristic 1-abaxial (dorsal) and 2-adaxial (ventral) relationship, whether the carpels are apocarpous or syncarpous to various degrees and whether the vasculature is amplified by additional bundles or not, is extraordinarily widespread. In *Nelumbo* (Leeuwen, 1963), for example, where many bundles sweep into each carpel



FIGS. 1–5. Fig. 1. *Salix alba.* Median longitudinal section of a female flower showing a nectary without a vascular supply. Fig. 2 *Salix safsaf.* Sketch of a male flower showing a structure intermediate between a corolla and the nectaries found in many other species. Fig. 3. *Myristica fragrans.* Median longitudinal section of a female flower showing vascularization. Figs. 4, 5. *Crataeva religiosa* Fig. 4. Semi-diagrammatic representation of a transverse section of a typical bicarpellary ovary at about its middle where the locules are confluent. Fig. 5. Diagram of a tetracarpellary ovary about in its middle. (Figs. 1, 2, redrawn from Fisher, 1928, no magnification indicated. Fig. 3, redrawn from Sastri, 1959, X 18. Figs. 4, 5, redrawn from Puri, 1950, no magnifications indicated).

D, dorsal carpellary vascular bundle; I, intermediate structure between corolla and nectary; N, nectary; X, lateral strand (sensu Puri, 1950) or dorsal carpellary vascular bundles, either vestigial or to accessory carpel. V, ventral carpellary vascular bundle;

from the receptacle, the three principal bundles, two inverted, enter the base of each carpel, or arise from a common bundle at the base of each carpel, and assume characteristic distribution. There are those, of course, who question this fundamental character (as Lam, 1961; Meeuse, 1971; Melville, 1962); but this is, perhaps, because they have not recognized the following additional character.

(3) The two lateral (ventral) carpellary bundles are inverted to some degree. Aside from all of the arguments concerned with whether carpels are conduplicately or involutely sealed, modified appendages or organs *sui generis,* primitively peltate, leguminoid, or telomic, or even whether they are appendicular or something else, and whether the veins are constantly oriented throughout their lengths or not, the two ventral carpellary bundles, nevertheless, are *not* "normally" oriented (to my knowledge). They most commonly show a complete inversion, that is, they have become phylogenetically rotated within the confines of the carpels, largely, 180° from the "normal" orientation of the cauline, trace, and dorsal carpellary bundles. Although the bundles twist throughout their courses in the carpels, they rotate first clockwise, then counter-clockwise, never far from their 180° (or 90° in conduplicately folded carpels) reorientation.

In my opinon, these two aspects of floral anatomy, the demonstrable conservatism of the vascular systems in so many cases and the intrinsic (widespread, if not constant due to extreme modification) conservatism of the fundamental vasculature of the receptacle and carpels, compel me to question the rationality of any summary rejection, which permits no retraction, of the concept of conservatism of the vascular system of the flower. Since conservatism can be demonstrated clearly, it seems obvious that it does exist in many floral vascular systems. It is equally obvious that it does not always exist, and that its absence in many genera has been demonstrated. We have, therefore, those who, because conservatism is not always present or demonstrable, have naively decided that it does not exist at all and can be berated and ignored. This seems to be an untenable misunderstanding of the mutability and variability of organisms.

The ultimate use of floral anatomy in taxo-phylogenetic studies is one of interpretation. Essentially all studies terminate in interpretation, which must be intelligent, experienced, and judicious. Carlquist (1969) emphasizes this. The vascular system of a flower should only be used as any other morphological or anatomical character. What it appears to reveal to an investigator should generally agree with the other kinds of evidences available, such as cytotaxonomy, other anatomical features, gross comparative morphology, etc. Although floral anatomy may have misled us on occasions, I submit that the concept that floral vasculature is conservative has been vindicated many times and has been valuable more often than not in explaining what the evolution of a flower has been and in contributing to taxonomic and phylogenetic ideas. To this should be repeated (Moseley, 1967) my opinion that the floral vascular system is too valuable a source of phylogenetic information to ignore in any serious examination of a taxon for taxonomic or phylogenetic purposes, and an investigator does so at his own risk.

Two questions bearing on vascular conservatism raised by Carlquist (1969) bear comment. He asks why the vascular system of the flower is thought to be conservative. I believe that it is because many cases have been described where conservatism is the logical explanation. He also cites examples of conservatism: the delayed fusion of adjacent bundles following adnation of floral organs in Ericaceae (Hancy, 1916); the delayed fusion of vasular bundles of fused flowers in some species of *Lonicera* (Wilkinson, 1948); the series of variously fused adjacent bundles characteristic of the perianth members in Labiatae (Eames and MacDaniels, 1947). Carlquist considers these classical examples of the delayed fusion of bundles as the only true conservatism which occurs when there is no change of function. On the contrary, although I certainly consider these examples of conservatism, there are many other examples more positive and vindicating, a very few of which I have cited above.

Carlquist (1969) also stresses that the venation of carpels has been used by different writers to support different theories for the gynoecium of flowers (Eames, 1961; Melville, 1962; Meeuse, 1971; Saunders, 1937). To this are two obvious replies. (1) The same material has often been interpreted differently in biology; this seems to be characteristic of human-kind. (2) Although it becomes almost unpleasantly personal, many writers unfortunately have displayed an inexpert understanding of, not just floral vein anatomy, but the entire fundamental plant anatomy of axis, appendage, and flower, such as: lacunae and trace (nodal) anatomy, the orientation of vascular bundles, the differences between stele and vascular bundle or between axis and appendage.

It is my impression that many floral anatomists have assumed that functional-structural relationships in flowers exist although such relationships are not easily demonstrable. Although criticism for neglecting to consider functional and adaptational aspects is justified; I believe that in many cases a writer believes that the lack of demonstrability should not impede an investigation, expects that other anatomists and morphologists reading his work will understand the limitations and realize that the writer was not unaware of the pertinence of functional aspects. Many floral anatomists have included functional interpretations.

THE RELATIVE VALUES OF DIFFERENT CHARACTERS

Carlquist (1969) suggests in Principle 6 that external morphology of the flower and fruit are probably the most reliable criteria for understanding their phylesis. This may be true in individual cases, but I suggest in reply that this casts aside all of the gains we have made in phylogeny over the past five decades during which we have learned repeatedly that our understanding of phylogeny advances more rapidly and soundly when we encompass many lines of evidence in any study. My comment is influenced by the assumption that the study of floral phylesis and the origin and phylesis of angiosperms are inextricably interwoven.

The most thorough and comprehensive plant system ever composed, that of Engler and Prantl (1897–1915), was based principally on comparative gross (external) morphology. The system exists today in modern forms as the most inclusive and most utilized one, yet the most widely unacceptable one on phylogenetic grounds. Nearly all studies on xylem, phloem, floral and inflorescence anatomy (e.g., Abbe, 1935; Bailey, 1949, 1953; Fisher, 1928; Hall, 1952; Moseley, 1948; Thompson and Bailey, 1916; Tippo, 1938) have shown the impossibility of Amentiferae as

TABLE 1

CONDITION OF CERTAIN EXTRINSIC CHARACTERS IN SELECTED ANGIOSPERMOUS ORDERS AND FAMILIES

Orders and Families	Lengths of Imperforate Elements	Lengths of Vessel Elements	Type of Vessel Element Perforation	Sieve-tubes present or not ; Type sensu Zahur (1959)	Pollen Types, sensu Erdtman (1952)
MAGNOLIALES					
Amborellaceae (16, 23)	Range : 1730-4660 μ	No Vessels	Absent	Present	Non-aperturate
Austrobaileyaceae (7, 16, 22, 33)			Scalariform	Absent	1-sulcate
Canellaceae (16, 34, 42, 43, 46)	Most common range: 1500-2000 μ	Range : 900-2400 μ	Scalariform, some reticulation	Present, I-III	1-sulcate
Magnoliaceae (9, 10, 14, 22, 46)	Mean lengths : 1200-2300 μ	Mean lengths : 800-1100 μ	Largely scalariform, some simple	Present, I-II	1-sulcate
Winteraceae (3, 16, 21, 37)	Mean lengths : 4000-4300 μ (Up to 6300 μ)	No vessels	Absent	Present	Distally uniaperturate
SUMMARY FOR MAGNOLIALES	Range : 300-6300 μ	129-2400 μ (No vessels in 8 genera)	Predominantly scalariform, scalariform and simple, or simple	Present (except *Austrobaileya*), I-III	7 families have 1-sulcate, otherwise principally 3-sulcate or derivatives, or non-aperturate
Other families tabulated : Annonaceae, 11, 16, 22, 27, 41, 46 ; Calycanthaceae, 2, 12, 13, 16, 20, 38 ; Degeneriaceae, 6, 16, 34 ; Eupomatiaceae, 12, 16, 20, 22 ; Gormotegaceae, 32; Hernandiaceae, 16, 22 ; Himantandraceae, 5, 16, 22, 28, 46 ; Illiciaceae, 16, 19, 22, 34 ; Lactoridaceae, 16, 22, 34 ; Lauraceae, 16, 22, 31, 34, 46 ; Monimiaceae, 16, 20, 22, 23, 46 ; Myristicaceae 12, 45, 46 ; Schizandraceae, 4, 8, 16, 22, 29, 34 ; Trimeniaceae, 16, 23.					
SUMMARY FOR TROCHODENDRALES	Range : 2800-6000 μ	No vessels	Absent	Present	3-4 colpate or 3 colporate
Families Tabulated : Tetracentraceae, 16, 22, 26, 37 ; Trochodendraceae, 8, 16, 26, 37.					
RANUNCULALES					
Menispermaceae (16, 22, 34)	Mean lengths : 1100-1200 μ	Mean lengths : 300-4500 μ	Simple	Present	Mostly 3-colporate ; 2-3 colpate, or other
SUMMARY FOR RANUNCULALES	Mean lengths : 400-1200 μ	Mean lengths : 230-4500 μ	Scalariform or simple	Present, II, III	2-3 colpate and derivatives
Other Family Tabulated : Lardizabalaceae, 16, 20, 22, 34, 46.					
SUMMARY FOR HAMAMELIDALES	Mean lengths : 1200-2900 μ	Mean lengths : 760-1600 μ	Scalariform	Present, I or ?	3-colpate and derivatives
Famllies Tabulated : Cercidiphyllaceae, 16, 20, 21, 34, 46 ; Eupteleaceae, 16, 20, 22, 34 ; Hamamelidaceae, 16, 22, 38.					
DILLENIALES					
Dilleniaceae (15, 16, 46)	Range : 625-4375 μ	Range : 625-3074 μ	Predominantly scalariform, some simple	Present, III	3-colpate, 3-rupate, or 3-colporate

SUMMARY FOR DILLENIALES	Range : 160-4375 μ	Range : 97-3074 μ	Predominantly scalariform, scalariform and reticulate, scalariform and simple, or simple	Present, III	3-colpate and other types, no 1-sulcate
Other families tabulated : Crossosomataceae, 16, 22 ; Paeoniaceae, 16, 18 ; Tetraceraceae. 15, 16, 46.					
SUMMARY FOR PIPERALES	Mean lengths : 500-1986 μ	Mean lengths : 200-856 μ (No vessels in *Sarcandra*)	Scalariform to simple (Absent in *Sarcandra*)	Present, III	1-sulcate, or other
Families tabulated : Chloranthaceae, 16, 23, 35, 46 ; Piperaceae, 16, 22, 29, 46.					
ROSALES					
Hydrangeaceae (16, 22, 38)	Mean lengths : 1100-1900 μ	Average lengths : 560-1100 μ	Scalariform	Present	3-colporoidate
SUMMARY FOR ROSALES	Mean lengths : 500-2100 μ	Mean lengths : 193-1120 μ	Scalariform, scalariform and simple, or simple	Present, I-III	Several types, no 1-sulcate
Other Families Tabulated : Cunoniaceae, 16, 22, 28, 46 ; Escalloniaceae, 22, 38 : Grossulariaceae, 16, 22, 38 : Rosaceae, 16, 22, 38, 46.					
THEALES					
Theaceae (16, 22, 40, 46)	Mean lengths : 1200-3100 μ	Mean lengths : 1000-1600 μ	Scalariform	Present, II-III	3-colporate or 3- colporoidate
SUMMARY FOR THEALES	Mean lengths : 800-3100 μ	Mean lengths : 300-1600 μ	Predominantly simple, some scalariform	Present, II and III in Theaceae	3-colpate and derivatives
Other Families Tabulated : Hypericaceae, Guttiferae, Quiinaceae, Bonnetiaceae, Dipterocarpaceae, 16, 22, 40, 46.					
"AMENTIFERAE"					
Casuarinaceae (16, 24, 46)	Range : 224-2016 μ	Range : 190-1275 μ	Scalariform or scalariform and simple	Present II	2-5 porate, mostly 3
Fagaceae (16, 22, 38, 46)	Mean lengths : 600-1600 μ	Range of averages 290-576 μ	Scalariform and simple or simple	Present, II-III	2-7 colpate and derivatives
SUMMARY FOR "AMENTIFERAE"	Range : 210-2590 μ	Range : 20-1275 μ	Predominantly simple, others scalariform and simple or scalariform	Present, largely II and III, some I	2-7 colpate, colporate or porate or derivatives
Other Families Tabulated : Betulaceae and Corylaceae, 16, 17, 22 ; Eucommiaceae, 16, 39 ; Garryaceae, 16, 25 ; Juglandaceae, 16, 22, 38 ; Julianiaceae, 16, 30 ; Moraceae, 16, 22, 38, 46 ; Myricaceae, 1, 16, 22 ; Rhoipteleaceae, 16, 44, 46 ; Salicaceae, 16, 22 ; Ulmaceae, 16, 36 46 ; Urticaceae, 16, 22, 38, 46.					

Numbered citations in table refer to : 1, Abbe, 1963 ; 2, Bailey, 1960 ; 3, Bailey and Nast, 1943 ; 4, ——, 1948 ; 5, Bailey, Nast and Smith, 1943 ; 6, Bailey and Smith, 1942 ; 7, Bailey and Swamy, 1949 ; 8, Bailey and Tupper, 1918 ; 9, Canright, 1953 ; 10, ——, 1955 ; 11, ——, 1962 ; 12, ——, 1963 ; 13, Cheadle and Esau, 1958 ; 14, ——, and ——, 1964 ; 15, Dickison, 1967 ; 16, Erdtman, 1952 ; 17, Hall, 1952, ; 18, Keefe, 1968 ; 19, Lemesle, 1933 ; 20, ——, 1956, ; 21, Mclaughlin, 1933 ; 22, Metcalfe and Chalk, 1950 ; 23, Money, Bailey and Swamy, 1950 ; 24, Moseley, 1948 ; 25, Moseley and Beeks, 1955 ; 26, Nast and Bailey, 1945 ; 27, Periasamy and Swamy, 1959 ; 28, Record, 1936 ; 29, —— and Hess, 1943 ; 30, Stern, 1952 ; 31, ——, 1954 ; 32, ——, 1955 ; 33, Swamy, 1953 ; 34, —— and Bailey, 1949 ; 35, —— and ——, 1950 ; 36, Sweitzer, 1971 ; 37, Thompson and Bailey, 1916 ; 38, Tippo, 1938 ; 39, ——, 1940 ; 40, Vestal, 1937 ; 41, Walker, 1971 ; 42, Wilson, 1960 ; 43, ——, 1965 ; 44, Withner, 1941 ; 45, Wyk, Van der and Canright, 1956 ; 46, Zahur, 1959.

primitive angiosperms, and Coniferophyta and Gnetophyta as ancestors.

Granted that pollination and dispersal mechanisms (Faegri and Van Pijl, 1966; Grant, 1950) have strongly influenced angiospermous evolution, we must not again rely on one kind of evidence in an investigation. The solution is not to determine which kind of evidence should be used, but to increase the kinds of data to a maximum in all of our studies. We have also learned that, although some kinds of data are often more valuable than others, the relative value of one kind of character changes from taxon to taxon. How many times must we learn these lessons?

PRIMITIVENESS AND THE RANALES

Finally, I would like to discuss the primitiveness of the Ranales (*sensu lato* and *sensu* Eames, 1961), a problem raised by Carlquist (1969) in the discussion of his Principle 3. For many years, morphologists and others have felt certain that evidence had finally determined that Ranales were primitive dicotyledons (Bailey, 1949, 1953), or had the greatest assemblage of primitive features, and hence their unusual characters, such as unsealed conduplicate carpels with extensive stigmatic regions; planated, broad stamens; and laminal placentation of ovules between dorsal and ventral carpellary veins, have been considered primitive.

Carlquist (1969) on morphological grounds, and Kubitski (1969) and Meeuse (1970) on biochemical grounds, have challenged the "most primitive" status and ancestral concept accorded the Ranales. Leaving aside the matter of whether or not a taxon can be termed primitive (something we all understand), I would like to delve to the heart of the problem, as I see it. We cannot certainly determine whether a taxon of the angiosperms is primitive or not by examining characters which are restricted to, or intrinsic to, the angiosperms (e. g., apocarpy, polymery). This ends in fruitless circular reasoning and nineteenth centrury conclusions. Bailey (1949, 1953) has drawn our attention to a few morphological and anatomical characters (e.g., the reduction of the lengths of tracheary elements) whose general evolutionary trends were determined, so far as we can now determine, in taxa other than, and antecedent to, the angiosperms as extrinsic, unidirectional trends. They are effective at supra-divisional level in vascular plants. These trends have continued their evolution in the angiosperms; and, since the angiosperms are so variable, the same trends are also useful for comparing evolutionary levels among angiosperm taxa. In my opinon, with extermely few exceptions, no other morphological or anatomical trends are valid for determining the overall directions of angiosperm evolution. An exception is the type of vessel perforation plate, and this may be an extrinsic character. We need understanding of more trends of this sort than we now possess. I wish to show by use of three of these extrinsic trends, and the kind of perforation plate, that there is moderate substance to the current distrust of the Ranales as the *most primitive* of angiosperms (the manner of expression does convey a definite meaning), but demonstrate that they certainly should not yet be removed from that status.

The three extrinsic evolutionary trends utilized in Table 1 to compare those taxa suggested as primitive angiosperms are: the reduction of tracheary elements in length, the presence or absence of sieve-tubes, and the occurrence of 1—sulcate pollen grains. Table 1 is a summary of a more extensive tabulation (available on request) of the condition of the listed characters on the principal families of each order represented. With the exception of the Hamamelidales, which is restricted to the families listed, and the artificial "Amentiferae", the categories are the same as those of the same names in Cronquist (1968).

It was determined long ago that the prevailing trend of tracheary elements in vascular plants for secondary elements (Bailey and Tupper, 1918) and primary elements (Bailey, 1944; Henes, 1959) is toward reduction in their lengths. It has also been shown that, to date, only one angisoperm lacks sieve tubes (Bailey and Swamy, 1949). I have extended the Tabulation, when known, to the types of sieve-tube elements as recorded by Zahur (1959); however, this character probably does not represent an extrinsic trend. All three types listed by Zahur are sieve-tube elements with Type I the most primitive.

It has also been shown that 1—sulcate (1—colpate) pollen is characteristic of gymnosperms (Erdtman, 1952), and that, in dicotyledons, the trend is toward multisulcate, multiaperturate or non-aperturate pollen or their derivative types. 1—sulcate pollen is, then, a primitive character.

To the three basic trends, I have added a fourth, that of the type of vessel perforation plate since the concept that the scalariform perforation plate is primitive and the simple plate advanced in the angiosperms (Bailey, 1949, 1953; Frost, 1930) has not been successfully challenged to my knowledge. A further character, essentially an extrinsic one, hence valid, appears serendipitously: the absence of vessel elements in genera where there is no evidence that they have evolved (Bailey, 1949, 1953; Thompson and Bailey, 1916).

It can be seen immediately, from examination of Table 1, that the longest imperforate tracheary elements clearly occur in the Winteraceae of the Magnoliales and the Trochodendrales, both ranalean taxa in the broad sense. The longest vessel elements occur in the Menispermaceae of the Ranunculales and the Canellaceae of the Magnoliales, again ranalean, and the Dilleniaceae of the Dilleniales. The occurrence of perforation plate types at familial level is less decisive. At ordinal level, however, scalariform perforation plates predominate only in the Magnoliales, the Hamamelidales (Eupteleaceae and Cercidiphyllaceae) and the Dilleniales. Scalariform plates appear as the only types in the Hydrangeaceae and Theaceae, mentioned by Carlquist (1969) as possible primitive angiosperms. Perhaps more important than the occurrence of primitive scalariform perforation plates is the primitive absence (Bailey, 1949, 1953; Thompson and Bailey, 1916) of perforation plates, hence vessels, in seven genera of Magnoliales, both genera of the Trochodendrales, and *Sarcandra* of Piperales. The complete absence of sieve tubes in *Austrobaileya* (Magnoliales) and the restriction of 1—sulcate pollen grains to Magnoliales and Piperales are strongly significant.

The predominace of scalariform plates and the relatively long imperforate and vessel elements give the Dilleniales some reason to be considered relatively primitive dicotyledons. Similarly, the absence of vessels in *Sarcandra* and the presence of 1—sulcate pollen in the Piperales, requires some consideration of the Piperales as a primitive taxon. The

Dilleniales, however, lack 1–sulcate pollen grains, all genera described have vessels (and the sieve-tubes are of the most advanced type); The Piperales have relatively short imperforate and vessel elements (and have the most advanced kind of sieve-tube elements). Neither Piperales nor Dilleniales express the primitive levels of so many of the extrinsic characters as do the Magnoliales or Trochodendrales. The Ranunculales (only woody members are considered) demonstrate a strongly primitive level of only one extrinsic character, the lengths of vessel elements. Similarly, the Hamamelidales (as listed in the table) demonstrate the primitive level of only one of the characters considered, the scalariform perforation plate (and this may not be an extrinsic charcter). The Hydrangeaceae and Theaceae, and the Rosales and Theales more generally, offer no evidence for the primitive assignment suggested by Carlquist (1969), except for the occurrence of exclusively scalariform vessel element perforation plates in each family. These are the only families in the two orders, respectively, which have exclusively scalariform perforation plates. The "Amentiferae" offer no challenge whatsoever, on the basis of Table 1, to the concept of the relative primitiveness of the Magnoliales and Trochodendrales, the principal woody ranalean orders.

It seems reasonable to claim, therefore, that the Magnoliales and Trochodendrales, at least on the basis of the most unbiased anatomical and morphological characters we have, are the most primitive dicotyledons (that is, these two orders possess among their members the greatest expression of the primitive levels of the extrinsic characters considered). The Dilleniales probably should be accorded a relatively low evolutionary level, but they are clearly more advanced than the Magnoliales and Trochodendrales, on the the basis of the characters considered, we do not yet have evidence of the very basic morphological and anatomical sort considered in this paper which permits replacing the Magnoliales and Trochodendrales as primitive dicotyledons by any other angiospermous taxon. At this moment, despite the intriguing suggestions of Kubitski (1969) and Meeuse (1970), the evidence strongly supports the widely held opinion that they are the most primitive existing angiospersms.

REFERENCES

ABBE, E. C. 1935. Studies in the phylogeny of the Betulaceae. I. Floral and inflorescence anatomy and morphology. *Bot. Gaz.* **97:** 1–67.

ABBE, L. B. 1963.X ylem anatomy of the Myricaceae. *Am. J. Bot.* **50:** 632–633.

ARBER, A. 1933. Floral anatomy and its morphological interpretation. *New Phytol.* **32:** 231–242.

BAILEY, I. W. 1944. The development of vessels in angiosperms and its significance in morphological research. *Am. J. Bot.* **31:** 421–428.

——. 1949. Origin of the angiosperms: need for a broadened outlook. *J. Arnold Arbor.* **30:** 64–70.

——. 1953. Origin of the tracheary tissue of land plants. *Am. J. Bot.* **40:** 4–8.

——.1960. Some useful techniques in the study and interpretation of pollen morphology. *J. Arnold Arbor.* **41:** 141–148.

——, AND C.G. NAST. 1944. The comparative morphology of the Winteraceae. III. Wood. *J. Arnold Arbor.* **25:** 97–103.

——, AND——. 1948. Morphology and relationships of *Illicium*, *Schisandra* and *Kadsura*. 1. Stem and leaf. *J. Arnold Arbor.* **29:** 77–89.

——, ——, AND A.C. SMITH. 1943. The family Himantandraceae *J. Arno Arbor.* **24:** 190–206

——, ——, AND ——. 1942. Degeneriaceae, a new family of flowering plants from Fiji. *J. Arnold Arbor* **23:** 356–365

——, AND B.G.L. SWAMY. 1949. The morphology and relationships of *Austrobaileya*. *J. Arnold Arbor.* **30:** 211–226

——, and W. W. Tupper. 1918. Size variation in tracheary cells I. A comparison between the secondary xylems of vascular cryptogam, gymnosperms, and angiosperms. *Proc. Am. Acad. Arts Sci.* **54:** 149–204.

Blaser, H. W. 1941. Studies in the morphology of the Cyperaceae. I Morphology of the flowers. A. Scirpoid genera. *Am. J. Bot.* **28:** 542–551.

Boothroyd, L. E 1930. The morphology and anatomy of the inflorescence and flower of the Platanaceae *Am. J. Bot.* **17:** 687–693.

Canright, J. E 1953 The comparative morphology and relationships of the Magnoliaceae—II. Significance of the pollen. *Phytomorphology* **3:** 355–365.

——. 1955. The comparative morphology and relationships of the Magnoliaceae IV. Wood and nodal anatomy. *J. Arnold Arbor.* **36:** 119–140.

——. 1962. Comparative morphology of pollen of Annonaceae. *Pollen Spores* **4:** 338.

——. 1963 Contributions of pollen morphology to the phylogeny of some ranalean famillies. *Grana Palynol* **4:** 64–72.

Carlquist, S. 1969. Toward acceptable evolutionary interpretations of floral anatomy. *Phytomorphology* **19:** 332–363.

Cheadle, V. I., and K. Esau. 1958. Secondary phloem of *Calycanthaceae*. *Univ. Calif. Publs. Bot.* **29:** 397–510.

——, and——. 1964. Secondary phloem of *Liriodendron tulipifera*. *Univ. Calif. Publs. Bot* **36:** 143–252

Cronquist, A. 1968. *The Evolution and Classification of Flowering Plants*. Houghton-Mifflin Co., Boston.

Dickison, W. C., 1967. Comparative morphological studies in Dilleniaceae. I. Wood anatomy. *J. Arnold Arbor.* **48:** 1–23.

Douglas, G.E. 1957. The inferior ovary. II *Bot. Rev* **23:** 1–46.

Eames, A. J. 1961. *Morphology of the Angiosperms*. McGraw-Hill Book Co. New York.

——, and L. H. MacDaniels 1947. *An Introduction to Plant Anatomy*. McGraw-Hill Book Co. New York.

Erdtman, G. 1952. *An Introduction to Palynology. I. Pollen Morphology and Plant Taxonomy. Angiosperms* Chronica Botanica Co. Waltham.

Engler, A., and K. Prantl. 1897–1915. *Die naturlichen Pflanzenfamilien*. 2nd. ed. 1924—incomplete. Leipzig.

Faegri, K., and L. van der. Pijl. 1966. *The Principles of Pollination Ecology*. Pergamon Press, London

Fisher, M. J. 1928. The morphology and anatomy of the flowers of the Salicaceae. I. *Am. J. Bot.* **15:** 307–326.

Frost, F. H. 1930. Specialization in secondary xylem of dicotyledons. II. Evolution of the end wall of vessel segment. *Bot. Gaz.* **90:** 198–212.

Grant, V. 1950. The protection of the ovules in flowering plants. *Evolution* **4:** 179–201.

Hall, J.W. 1952. The comparative anatomy and phylogeny of the Betulaceae. *Bot. Gaz.* **113:** 235–270.

Hancy, N.A. 1916. *Vascular Anatomy of Certain Ericaceous Flowers*. Ph. D. Thesis. Cornell Univ.

Henes, E. 1959. Fossile Wandstrukturen. In *Handbuch der Pflanzenanatomie*. W. Zimmermann, and P.G. Ozenda (eds.). 3 (5): 1–108.

Joshi, A. C. 1936. Vascular supply of the stamens of *Gloriosa superba*. *Nature, Lond.* **143:** 437.

Keefe, J. M. 1968. *The Comparative Anatomy, Morphology, and Phylogeny of Paeonia*. M.A. Thesis, Univ. Calif. Santa Barbara.

Koch, M. F. 1930. Studies in the anatomy and morphology of the composite flower. I. The corolla. *Am. J. Bot.* **17:** 938–952.

Kubitzki, K. 1969. Chemosystematischen Betrachtungen zur Grossgliederungder Didotylen. *Taxon* **18:** 360–368.

Lam, H. J. 1952. L' evolution des plantes vasculaires. *Annls Biol.* **28:** C57–C88.

——. 1961. Reflections on angiosperm phylogeny. II. Facts and Theories. *Koninkl. Nederl. Akad. Wetensch.—Amsterdam. Proc. Ser. C.* **64:** 263–276.

Leeuwen, W. A. M., van. 1963. A study of the structure of the gynoecium of *Nelumbo lutea* (Willd.) Pers. *Acta bot. neerl.* **12:** 84–97.

Lemesle, R. 1933. De l'anciennete des caracteres anatomiques des Magnoliacées. *Revue gèn. Bot.* **45:** 341–355.

——. 1956. Les èlèments de xylème dans les Angiospermes a caracteres primitifs. *Bull. Soc. bot.* **103:** 629–677.

McLaughlin, R. P. 1933. Systematic anatomy of the woods of the Magnoliales. *Trop. Woods* **34:** 3–37.

Meeuse, A. D. J. 1970. The descent of the flowering plants in the light of new evidence from phytochemistry. I. General discussion. *Acta bot. neerl.* **19:** 61–72.

——. 1971. Interpretative gynoecial morphology of the Lactoridaceae and the Winteraceae—a re-assessment. *Acta bot. neerl.* **20:** 221–238.

Melville, R. 1962. A new theory of the angiosperm flower. I. The gynoecium. *Kew Bull.* **16:** 1-50.

Metcalfe C. R., and L. Chalk 1950. *Anatomy of the Dicotyledons*. Vols. I, II. Oxford Univ. Press, Oxford.

Money, L. L., I. W. Bailey, and B. G. L. Swamy, 1950. The morphology and relationships of the Monimiaceae. *J. Arnold Arbor.* **31:** 372-404.

Moore, J. M. 1936. The vascular anatomy of the flower in the papilionaceous Leguminosae. I. *Am. J. Bot.* **23:** 279-290.

Moseley, M. F. 1948. Comparative anatomy and phylogeny of the Casuarinaceae. *Bot. Gaz.* **110:** 231-280.

——. 1967. The value of the vascular system in the study of the flower. *Phytomorphology* **17:** 159-164.

——, and R. M. Beeks. 1955. Studies of the Garryaceae I. The comparative morphology and phylogeny. *Phytomorphology.* **5:**314-346.

NAST, C. G., AND I. W. BAILEY. 1945. Morphology and relationships of *Trochodendron* and *Tetracentron*. II. inflorescence, flower and fruit. *J. Arnold Arbor.* **26**: 267-276.

PERIASAMY, K., AND B. G. L. SWAMY. 1959. Studies in the Annonaceae —I. Microsporogenesis in *Cananga odorata* and *Miliusa wightiana*. *Phytomorphology*. **9**: 251-263.

PURI, V. 1950. Studies in floral anatomy. VI. Vascular anatomy of the flower of *Crataeva religiosa* with special reference to the nature of the carpels in the Capparidaceae. *Am. J. Bot.* **37**: 363-370.

——. 1951. The role of floral anatomy in the solution of morphological problems. *Bot. Rev.* **17**: 471-553.

RECORD, S. J. 1936. Classifications of various anatomical features of dicotyledonous woods. *Trop. Woods* **47**: 12-27.

——, AND R. W. HESS. 1943. *Timbers of the New World.* Yale Univ. Press, New Haven.

SASTRI, R. L. N. 1959. Vascularization of the carpel in *Myristica fragrans*. *Bot. Gaz.* **121**: 92-95.

SAUNDERS, E. R. 1937. *Floral Morphology*. W. Heffer and Sons Cambridge.

SMITH, G. H. 1926. Vascular anatomy of ranalian flowers. I. Ranunculaceae. *Bot. Gaz.* **82**: 1-29.

STERN, W. L., 1952. The comparative anatomy of the xylem and the phylogeny of the Julianiaceae. *Am. J. Bot.* **39**: 220-229.

——. 1954. Comparative anatomy and phylogeny of Lauraceae. *Trop. Woods* **100**: 1-72.

——. 1955. Xylem anatomy and relationships of Gomortegaceae. *Am. J. Bot.* **42**: 874-885.

SWAMY, B. G. L. 1953. The morphology and relationships of the Chloranthaceae. *J. Arnold Arbor.* **34**: 375-408.

——, AND BAILEY, I. W. 1949. The morphology and relationships of *Cercidiphyllum*. *J. Arnold Arbor.* **30**: 187-210.

——, AND ——. 1950. *Sarcandra*, a vesselless genus of the Chloranthaceae. *J. Arnold Arbor.* **31**: 117-129.

SWEITZER, E. M. 1971. Comparative anatomy of Ulmaceae. *J. Arnold Arbor.* **52**: 523-585.

THOMPSON, W. P., AND I. W. BAILEY. 1916. Are *Tetracentron*, *Trochodendron* and *Drimys* specialized or primitive types ? *Mem. N.Y. bot. Gdn.* **6**: 27-32.

TIPPO. O. 1938. Comparative anatomy of the Moraceae and their presumed allies. *Bot. Gaz.* **100**: 1-99.

——. 1940. The comparative anatomy of the secondary xylem and the phylogeny of the Eucommiaceae. *Am. J. Bot.* **27**: 832-838.

TROLL, W. 1933. Beiträge zur Morphologie des Gynaeceums IV. Über das Gynaeceum der Nymphaeaceen. *Planta* **21**: 447-485.

VESTAL, P. A. 1937. The significance of comparative anatomy in establishing the relationship of the Hypericaceae to the Guttiferae and their allies. *Philipp. J. Sci.* **64**: 199-256.

WALKER, J. W. 1971. Pollen morphology, phytogeography, and phylogeny of the Annonaceae. *Contr. Gray Herb. Harv.* No. **202**: 3-130.

WILKINSON, A. M. 1948. Floral anatomy and morphology of some species of the Tribe Lonicereae of the Caprifoliaceae. *Am. J. Bot.* **35**: 261-271.

WILSON, T. K. 1960. The comparative morphology of the Canellaceae. I. Synopsis of the genera and wood anatomy. *Trop. Woods* **112**: 1-27.

——. 1965. The comparative morphology of the Canellaceae. II. Anatomy of the young stem and node. *Am. J. Bot.* **52**: 369-378.

WITHNER, C. L. 1941. Stem anatomy and phylogeny of the Rhoipteleaceae. *Am. J. Bot.* **28**: 872-878.

WYK, R.W., VAN DER, AND J. E. CANRIGHT, 1956. The anatomy and relationships of the Annonaceae. *Trop. Woods* **104**: 1-24.

ZAHUR, M. S. 1959. Comparative study of secondary phloem of 423 species of woody dicotyledons belonging to 85 families. *Agric. Experiment Sta. Cornell Univ. Mem.* **358**: 1-160.

Adv. Pl. Morph. 1972: 408–416

STUDIES ON THE FLORAL ANATOMY, EVOLUTION OF THE GYNOECIUM AND RELATIONSHIPS OF THE FAMILY LOGANIACEAE

By Y. D. Tiagi and Shashikala Kshetrapal

Botany Department, Jiwaji University, Gwalior and Rajasthan University, Jaipur

Abstract

Floral anatomy of *Mitreola oldenlandioides, M. paniculata, Buddleia asiatica, Nicodemia diversifolia* and *Strychnos nux-vomica* has been investigated.

The flower is pentamerous in *Mitreola* and *Strychnos*. In *Buddleia*, both pentamerous and tetramerous flowers occur; *Nicodemia* has tetramerous flowers. The gynoecium usually consists of two laterally placed united carpels. Placentation is axile in the lower part but is parietal in the upper part of the ovary. Tricarpellary gynoecia, with the odd member on the posterior or anterior side have been observed occasionally in *Buddleia* and *Strychnos*. In *Buddleia*, tetracarpellary gynoecia have been commonly observed. In such cases, usually two of the carpels are median and the other lateral in position; rarely they are diagonal in position. Pentacarpellary gynoecia, usually with the odd member on the posterior side, rarely on the anterior side have also been observed in *Buddleia*. In all the genera, the ovary is superior, except in *Mitreola* wherein the ovary is more than half-inferior.

The sepals, petals, stamens and carpels are respectively, 3-traced, 1 or 3-traced, 1-traced and multitraced. In *Strychnos* and *Nicodemia*, the traces of the different floral cycles arise independently from the receptacular stele; in *Buddleia*, the traces of the sepals, petals and stamens arise conjointly and in *Mitreola* wherein the ovary is more than half-inferior, the vascular traces of all the floral cycles are conjointed at their origin.

Present studies indicate that the ancestral condition of the loganiaceous gynoecium was pentacarpellary and that the loss of one, two or three carpels has given rise, respectively to the tetra-, tri— and the bicarpellary condition. From a tetracarpellary gynoecium, such as is occasionally seen in *Buddleia asiatica*, the loss of the two median carpels would give rise to a gynoecium consisting of two laterally placed carpels and vice-versa. This explains satisfactorily the lateral or median placement of the two carpels of the gynoecium in the Loganiaceae.

Present studies indicate that the raising of the two subfamilies of the Loganiaceae to full-fledged families is not very sound. Loganiaceae is considered as a comparatively primitive taxon among the Metachlamydaeae, as one offering points of contacts with the Gentianales via taxa like *Logania*, with the Rubiales, *via* taxa like *Mitreola* and with the Scrophulariales via taxa like *Buddleia*.

Introduction

Loganiaceae is a small family, comprising about 35 genera and 550 species, occurring in the Tropical and Warm Temperate regions of the world. They are plants of varied habits, shrubs, trees, climbers and herbs. The relationships of the family have been a highly debated topic (Wettstein, 1935; Hutchinson, 1959; Takhtajan, 1966). There is practically no earlier work on the floral anatomy of this family. The present writers, during a preliminary work on the floral anatomy of certain locally available materials of this family observed a number of structural features of evolutionay interest. This prompted them to undertake this investigation.

MATERIAL AND METHOD

In the present work, floral anatomy of the following taxa has been investigated:

Taxon	Place of collection
Mitreola oldenlandioides Wall.	Sagar
Mitreola paniculata Wall.	Utrecht
Buddleia asiatica Lour.	Musoorie, Jaipur
Nicodemia diversifolia Tenore	Sagar
Strychnos nux-vomica Linn.	Rewa

Customary methods of microtechnique were employed. Serial transverse and longitudinal sections of the flower buds were cut at thickness ranging from 12-15. μ. Double staining with crystal violet and erythrosin gave satisfactory results.

OBSERVATIONS

Mitreola oldenlandioides Wall and M. paniculata Wall.—The bract is 1—traced (Fig. 1, A). A transverse section of the pedicel contains a ring of xylem which is enveloped on the outside and inside by phloem. At the base of the inferior ovary, five antisepalous and alternating with these five antipetalous vascular cords diverge out from the receptacular stele (Fig. 1B). These traverse upward inside the wall of the inferior ovary. The remaining receptacular stele becomes completely used up in the organization of the two compound carpellary marginal traces which traverse upward into the carpellary septum. Inside the wall of the inferior ovary, the ten bundles undergo a tangential splitting to form two concentric rings of ten vascular traces each (Fig. 1B, C). Of the ten bundles of the outer ring, five are the dorsal traces and the other five the compound marginal traces of the sepals. All the ten vascular traces of the inner ring are compound. On their inner side, they give off an indefinite number of carpellary traces. After this, five of them become the dorsal traces of the petals and the other five alternating with them become the five staminal traces (Fig. 1D, E). The sepals are, therefore, 3—traced, petal and stamen 1—traced and the carpels multitraced. The two carpels are laterally placed (Fig. 1D, G). The marginal bundles ramify inside the placentae, furnishing a single trace to each ovule (Fig. 1H, I). All the other carpellary traces, including even the carpellary dorsal bundles terminate blindly below the top of the ovary. The upper part of the ovary, styles and the stigmas are completely devoid of any vascular supply (Fig. 1G, H).

Buddleia asiatica Lour.—The flower is usually tetramerous. The two carpels are laterally arranged. In the Mussoorie material, a tricarpellary gynoecium with the odd member on the posterior side is occasionally observed (Fig. 1T). In the Jaipur material, both tetra—and pentamerous flowers are common but the usual condition of the gynoecium is tetracarpellary. Usually, two of the four carpels are median and the other two lateral in position (Fig. 1U). Occasionally, the four carpels have a diagonal disposition. Pentamerous flowers with penta—, tri—and even bicarpellary gynoecia also occur (Fig. 1V).

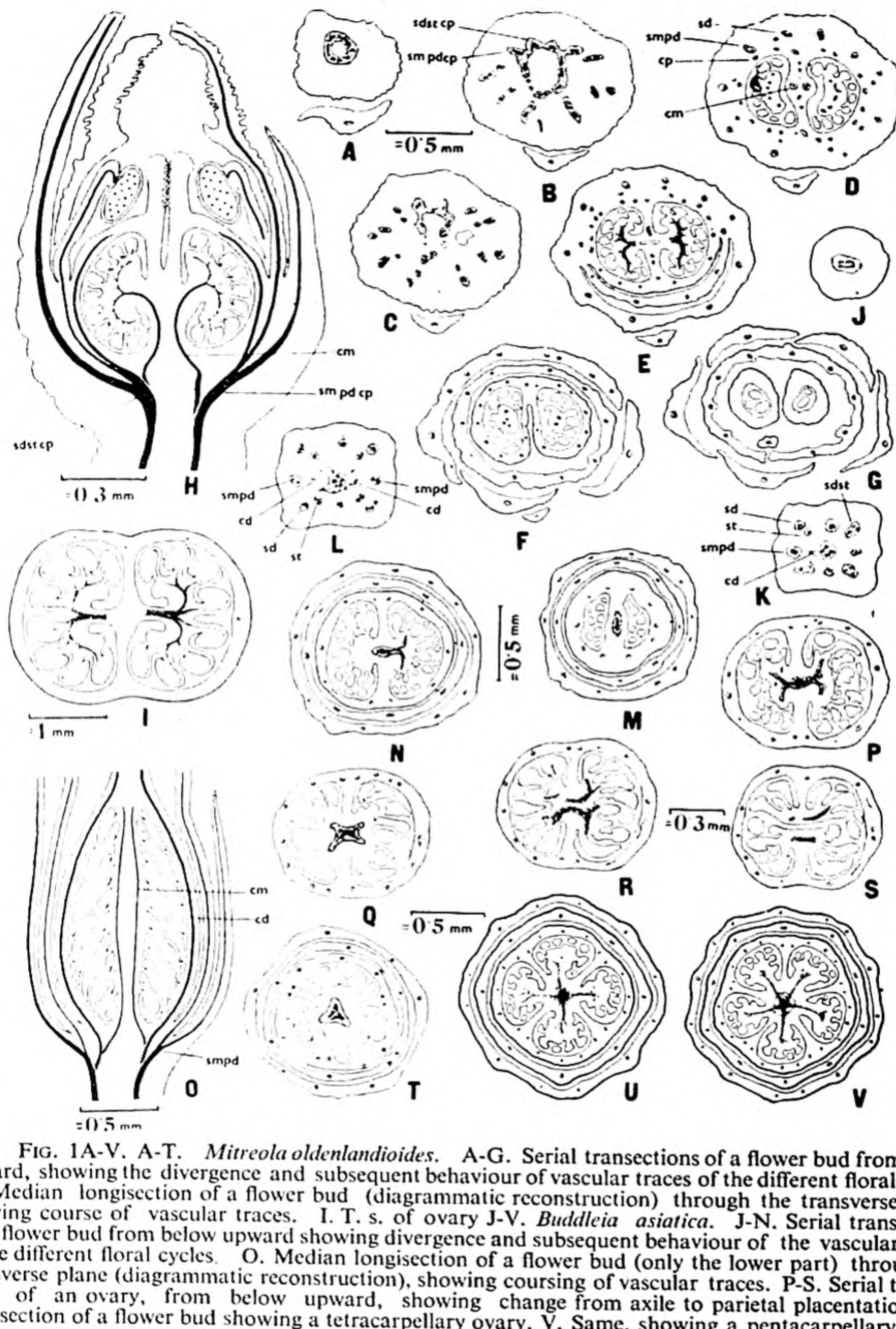

sdst cp
sm pdcp
=0.5 mm
A
B
sd
smpd
cp
cm
D
C
E
J
cm
sm pd cp
sdst cp
=0.3 mm
H
smpd
smpd
cd
cd
sd
st
L
F
G
sdst
sd
st
smpd
cd
K
I
=1 mm
N
=0.5 mm
M
P
Q
R
=0.3 mm
S
cm
cd
=0.5 mm
smpd
O
=0.5 mm
T
U
V

FIG. 1A-V. A-T. *Mitreola oldenlandioides*. A-G. Serial transections of a flower bud from below upward, showing the divergence and subsequent behaviour of vascular traces of the different floral cycles. H. Median longisection of a flower bud (diagrammatic reconstruction) through the transverse plane, showing course of vascular traces. I. T. s. of ovary J-V. *Buddleia asiatica*. J-N. Serial transections of a flower bud from below upward showing divergence and subsequent behaviour of the vascular traces of the different floral cycles. O. Median longisection of a flower bud (only the lower part) through the transverse plane (diagrammatic reconstruction), showing coursing of vascular traces. P-S. Serial transections of an ovary, from below upward, showing change from axile to parietal placentation. U. Transection of a flower bud showing a tetracarpellary ovary. V. Same, showing a pentacarpellary ovary.

cd, carpellary dorsal; cm, compound carpellary marginal; sd, sepal dorsal; sdst, compound trace of sepal dorsal and of a stamen; sdstcp, compound trace of sepal dorsal, of a stamen and carpel; smpdcp, compound trace consisting of sepal marginals, petal dorsal and a carpellary trace; st, staminal trace.

A transverse section of the pedicel contains an ectophloic siphonostele (Fig. 1J). In the apical region, both the receptacle, as well as its stele become almost quadrangular. Eight vascular cords now diverge out (Fig. 1K). These divide tangentially once and give rise to an outer ring of four dorsal traces and four compound marginal traces of the sepals and an inner ring of four dorsal traces of the petals and four staminal traces (Fig. 1L). The remaining stele is completely consumed up in furnishing one dorsal and four secondary marginal traces to each carpel. The remaining receptacular stele forms a mass, representing the four fused marginal traces of the two carpels (Fig. 1M-Q). In the lower part, the ovary is bilocular but during its upward course, the decompound marginal bundle splits up transversely, soon followed by the separation and slight outward recession of the two placentae making the ovary unilocular and placentation parietal (Fig. 1 Q-S). Only the dorsal bundles supply the style and stigma. Notwithstanding the ramifications of the marginal bundles inside the placentae, no vascular traces are furnished to the ovules (Fig. 1O-P, R).

Nicodemia diversifolia Tenore.—The floral plan of this species is very much similar to that of *Buddleia asiatica*, described earlier. Its vascular anatomy is also similar to that of *Buddleia* except that the vascular traces of the different floral cycles have an independent origin from the receptacular stele (Fig. 2A-H).

Strychnos nux-vomiva Linn.-The flowers are pentamerous. Each has two lateral bracteoles. The gynoecium consists of two laterally placed carpels. In rare cases, there are three carpels of which the odd member is on the posterior side (Fig. 2S). The pedicel contains a dissected amphiphloic siphonostele (Fig. 2I). A single trace is given out to a bracteole on each lateral side (Fig. 2J). Both sepals and petals are 3—traced in this species; stamens are 1—traced and carpels multitraced. The traces of the different floral cycles arise independently from the receptacular stele (Fig. 2K-R). As in the preceding species, the gynoecium is bilocular with axile placentation in the lower region but becomes unilocular with parietal placentation in the upper region (Fig. 2 P-O). Only the carpellary dorsal bundles supply the style and stigma (Fig. 2 Q-R).

DISCUSSION

Vascular ground plan and its Specialization.—A comparative study of the vascular anatomy of the flower in the Loganiaceae reveals, for the convenience of description, at least three stages of specialization. In *Strychnos nux-vomica*, the traces of the different floral cycles have an independent origin and there is no adnation among them. The condition in *Nicodemia* is more or less similar except that the dorsal trace of the posterior petal may, in some cases, arise conjointly with the nearby marginal traces of the sepals. *Buddleia* represents the next stage of specialization. Here, the dorsal traces of the sepals arise conjointly with the staminal traces and the dorsal traces of the petals arise conjointly with the marginal traces of the sepals. In spite of adnation at the point of origin of the traces of the sepals, petals and stamens, these organs are totally free from one another. *Mitreola* represents the final stage in this series of increasing specialization. In this genus, the sepals, petals, stamens and the carpels are all fused for about half of the height of the ovary

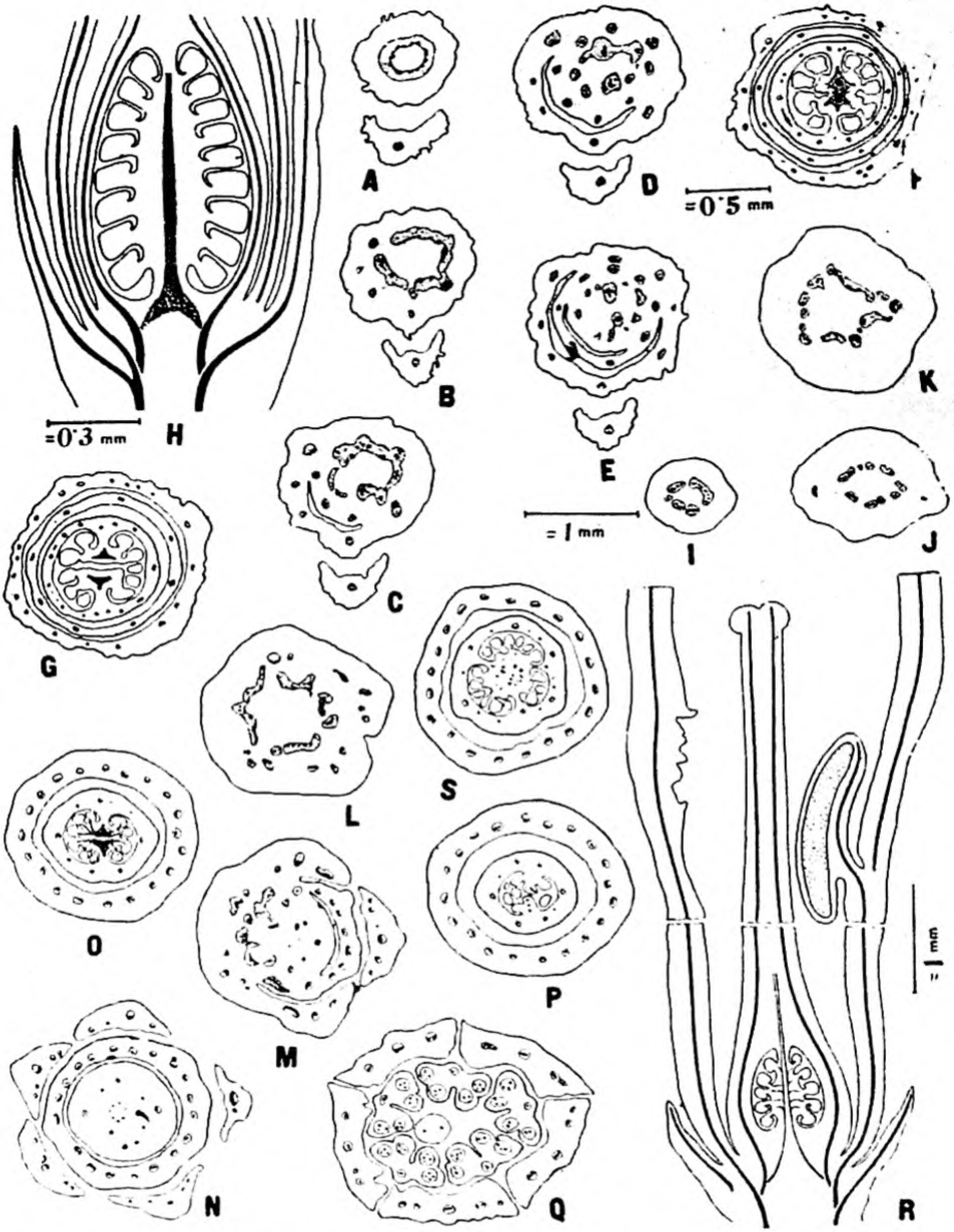


FIG. 2 A-R. A-H- *Nicodemia diversifolia*. A-G. Serial transections of a flower bud from below upward showing the mode of divergence and subsequent behaviour of the traces of the different floral cycles. H. Median longisection of a flower bud (lower part only) through the transverse plane (diagrammatic reconstruction), showing coursing of vascular traces. I-R. *Strychnos nux-vomica*. I-Q. Serial transections of a flower bud from below upward showing the divergence and subsequent behaviour of the traces of the different floral cycles. R. Median longisection of a flower bud (middle part removed) through the transverse plane (diagrammatic reconstruction) showing course of vascular traces.

which is slightly more than half-inferior. There is extreme adnation of traces of all the floral cycles. Thus in the family Loganiaceae, the course of evolution of the vascular skeleton of the flower has been from a condition where there is no adnation to a condition of complete adnation among the vascular traces of the different floral cycles.

Evolution of the gynoecium.—Present studies also shed a flood of light on the course of gynoecial evolution in the Loganiaceae. The genus *Buddleia* is the repository of much useful information in this connection. The following variation is observed in this taxon, as far as the number and position of carpels, constituting the gynoecium is concerned:

1. Usually, the gynoecium consists of two laterally placed carpels.
2. Tetramerous flowers, with tetracarpellary and tricarpellary gynoecia are also occasionally observed.
3. Pentamerous flowers, with pentacarpellary, tetracarpellary and tricarpellary gynoecia are also occasionally observed.
4. When the gynoecium consists of five carpels, these are usually antisepalous in position.
5. When the gynoecium is tetracarpellary, usually , two of the four carpels are median and the other two lateral in position. In several cases, however, they have been observed to be diagonally situated.
6. When the gynoecium consists of three carpels, the odd member has been observed to be situated, either on the posterior or the anterior side.

In all the material which has been studied in the present investigation the two carpels have been found to be laterally placed. Only very rarely, in a cymule, the two carpels in the median flower are medianly placed while in the lateral flowers, they are laterally placed. Rendle (1938) says that the two carpels comprising the gynoecium are generally median, rarely obique or transverse.

In *Buddleia, Nicodemia* and *Strychnos*, the ovary is bilocular in the lower region. All the four marginal bundles of the two carpels may be fused into a single decompound marginal bundle or there may be two compound marginal bundles, each formed of the fusion of the two marginal bundles of the same carpel. As one proceeds upward, the marginal bundles undergo a realignment, the adjacent marginal bundles of the different carpels fusing together, soon followed by the splitting and slight outward recession of the placentae. The ovary thus becomes unilocular and the axile placentation of its lower part gives rise to the truly parietal placentation of the upper part.

The above mentioned observations lead us to formulate the following course of evoiution for the gynoecium of the family Loganiaceae. The ancestral condition of the gynoecium in this family is believed to be pentacarpellary. All the other variants are supposed to have originated by the suppression of one or more carpels. Loss of one carpel from the anterior side would give rise to the tetracarpellary condition wherein two of the four carpels are median and the other two lateral in position; loss of the posterior carpel would leave four carpels, diagonally arranged. From a tetracarpellary condition, loss of the anterior or the posterior carpel would give rise to the tricarpellary condition in which the odd member is respectively on the posterior or the anterior side. Also from a tetracarpellary condition wherein the two carpels are median and two lateral, the loss of the former or the latter would give rise to a bicarpellary condition in which the two carpels are respectively lateral or median in position.

The change from axile to parietal placentation as one moves from the lower to the upper part of the ovary in the Loganiaceae is quite in conformity with the theory of the origin of parietal from axile placentation, as proposed by Puri (1952).

The above described ideas about the gynoecial evolution in the Loganiaceae are shown in Fig. 3.

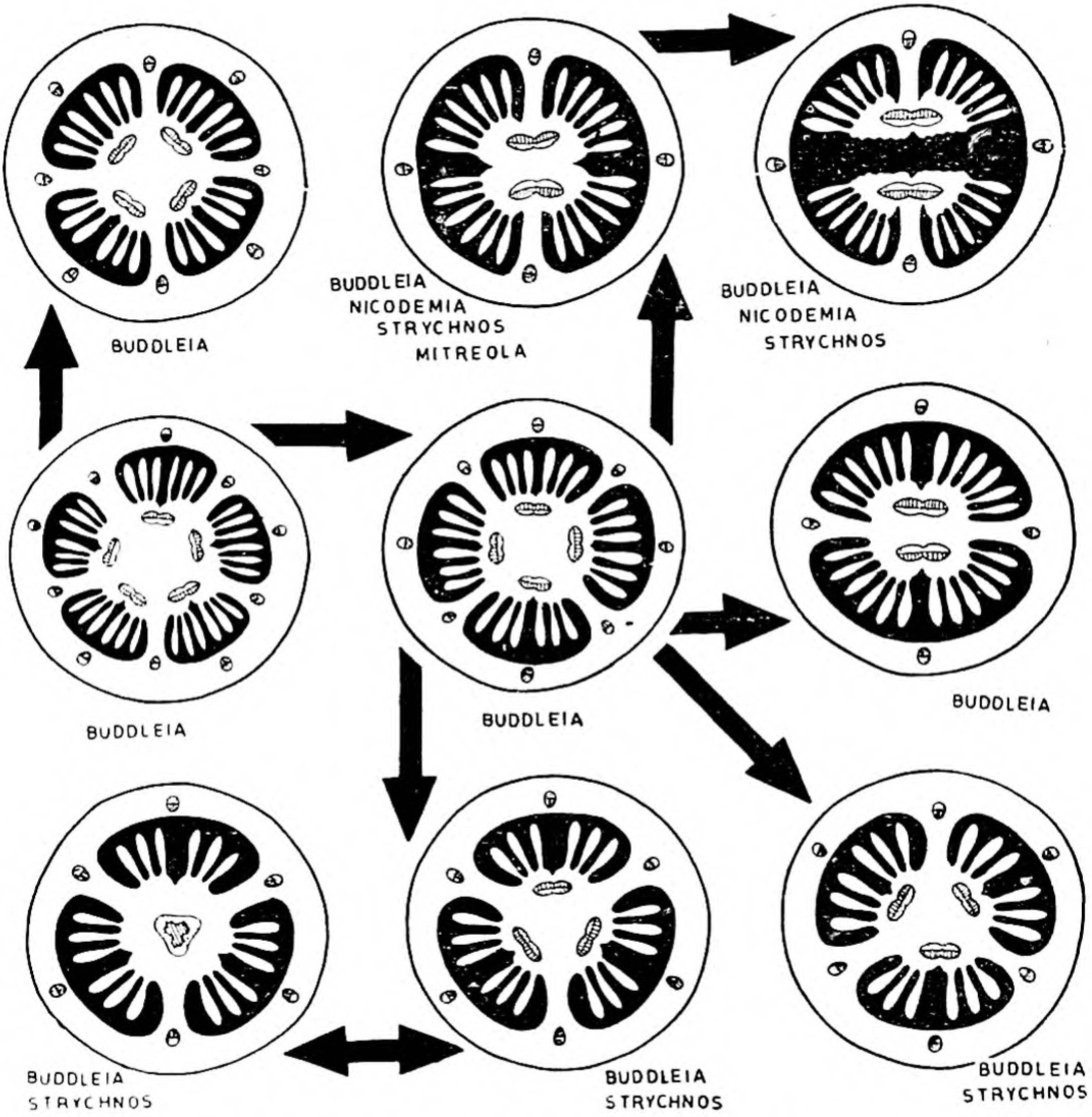

BUDDLEIA
BUDDLEIA
NICODEMIA
STRYCHNOS
MITREOLA
BUDDLEIA
NICODEMIA
STRYCHNOS
BUDDLEIA
BUDDLEIA
BUDDLEIA
BUDDLEIA
STRYCHNOS
BUDDLEIA
STRYCHNOS
BUDDLEIA
STRYCHNOS

FIG. 3. Diagrams of transections of ovaries of different taxa studied, showing course of gynoecial evolution in the Loganiaceae.

Phylogenetic considerations—The main problems are—how close is the relationship between the two subfamilies, viz., Loganioideae and Buddleioideae and to what other group, the Loganiaceae are related. It may be stated here that the Loganiaceae are genuine members of the Gentianales (Kshetrapal, 1967). The present discussion is concerned with their relationships out side the Gentianales.

The subfamily Loganioideae are characterized by the presence of intraxylary phloem, cork of superficial origin and lack of glandular and stellate hairs; the Buddleioideae are characterized by the lack of intraxylary phloem but have cork of pericyclic origin and stellate and glandular hairs. In view of these differences, a number of phylogenists have not only given full family status to both the subfamilies but some have kept them in widely separated groups. Hallier (1905) places the Loganiaceae in his Tubiflorae. Hutchinson (1959) gives full family rank to both the subfamilies and together with the Oleaceae keeps them under the order Loganiales. Wettstein (1935) indicates relationships of the Buddlejaceae with the higher Tubiflorae. According to Takhatajan (1966), the family Buddlejaceae with its toothed leaves and tetramerous flowers really remind some of the higher Laminiae, specially the Scrophulariaceae. Bentham (1857), Solereder (1895) and Klett (1924) have all pointed out the relationships of the genus *Buddleia* with the Scrophulariaceae. Solereder (1895) differentiated *Buddleia* from the Scrophulariaceae only on the basis of the presence of stipules in this genus which are absent in the Scrophulariaceae. Dop (1913) and Souegès (1940), on the basis of their embryological studies also concluded that *Buddleia* is closely related to the Scrophulariaceae. Loganiaceous genera like *Nuxia* (Africa and the Mascarene islands) and *Sanango* (Peru) have been kept at times in the Scrophulariaceae (Takhtajan, 1966).

In spite of the fact that the two subfamilies, the Loganioideae and the Buddleioideae differ from each other in some respects, the quantum of resemblance between them appears to be of a higher order than the differences. The Loganiaceae are mainly arborescent, have interpetiolar stipules, supplied by traces from the marginal bundles of the leaf or a stipular line; flowers are actinomorphic; staminal filaments are of equal length; gynoecium consists of 2 to 5 carpels; when bicarpellary, the 2 carpels are usually lateral in position; a hypogynous nectar disc below the ovary is lacking. On the contrary, the Scrophulariaceae are mainly herbaceous, have zygomorphic flowers; stamens four, didynamous, occasionally with a staminode on the posterior side; gynoecium consists of two, always medianly placed carpels.

In view of the above discussion, the inclusion of the Buddlejaceae under the Scrophulariales (Takhtajan, 1966) or the Tubflorales (Hallier, 1905) does not appear to be logical. The maximum which can be conceded is that the Buddleioideae offers a point of contact between the Loganiaceae and the Scrophulariaceae. Moreover, the differences between the Loganioideae and the Buddleioideae are well expressed by keeping them as such, as subfamilies of the Loganiaceae; raising them to the status of full-fledged families seems unwarranted. Further, in the opposite, decussate arrangement of the leaves; presence of interpetiolar stipules, supplied by vascular traces from the marginal bundles of the leaves; pentamerous or tetramerous, actinomorphic flowers; stamens of equal size, equal in number and alternating with the petals; gynoecium consisting of 2 to 5 carpels; when bicarpellary, the two carpels median or lateral in position; placentae T-shaped in cross sections and the almost inferior ovary in genera like *Mitreola*, the Loganiaceae offer a point of contact with the subfamily Cinchonoideae of the Rubiaceae. Gupta (1963) has investigated the floral anatomy of the family Rubiaceae. We find that the complete adnation of the vascular traces of the sepals, petals, stamens and carpels at the point of their origin in the genus *Mitreola* is remarkably similar to that seen in the Rubiaceae. An additional point which corroborates this affinity is the recent reporting of glandular squamellae in certain taxa of the Rubiaceae (Pandey, 1967) which are similar to those seen in several species of the Loganiaceae (Kshetrapal, 1967).

REFERENCES

BENTHAM, G. 1857. Notes on Loganiaceae. *J. Linn. Soc. Bot.* **1:** 52–114.

*DOP, P. 1913. Recherches sur le developement et la nutrition du sac embryonaire et de l'endosperm des *Buddleia*. *Bull. Soc. bot. Fr.* **60:** 9–16, 45–50, 92–98.

GUPTA, D.P. 1963. *Morphological and Anatomical Studies in the Family Rubiaceae*. Ph.D. Thesis, Agra Univ., Agra, India.

HALLIER, H. 1905. Provisional scheme of the natural (Phylogenetic) system of flowering plants. *New Phytol.* **4:** 151–162.

HUTCHINSON, J. 1959. The *Families of Flowering Plants.* Vol. I. Dicotyledons, (Second ed.). Oxford.

*KLETT, W. 1924. Umfang and Ihnalt der familie der Loganiaceen. *Bot. Archiv.* **5:** 312–338.

KSHETRAPAL, SHASHIKALA. 1967. *Morphological Studies in the order Gentianales*. Ph. D. Thesis, Sagar Univ., Sagar, India.

PANDEY, DEVENDRA. 1967. *Studies in the order Rubiales I.A Contribution to the Morphology and Vascular Anatomy of the Node and Flower of Certain Species of the Family Rubiaceae.* M.Sc. Thesis, Sagar Univ., Sagar, India.

PURI, V. 1952. Placentation in Angiosperms. *Bot. Rev.* **18:** 603-651.

RENDLE, A. B. 1938. *The Classification of Flowering Plants.* Vol. II. Dicotyledons. Cambridge University, Cambridge.

SOLEREDER, H. 1895. Loganiaceae. In Engler and Prantl. *Die Natürlichen Pflanzenfamilien.* Vol. IV. 19-30.

SOUEGES, R. 1940. Embryogenie des Loganiaceae. Development de l'embryon chez le *Buddleia variabilis* Hemsle. *J. C. r. Acad. Sci., Paris* **211:** 130-140.

TAKHTAJAN, A.L. 1966. *Systema et Phylogenia Magnoliophytorum* (in Russian). Moscow and Leningrad.

WETTSTEIN, R. 1935. *Handbuch der Systematischen Botanik*. IV ed. Leipzig and Wien.

---

* not seen in original

Adv. Pl. Morph. 1972: 417–422

THE GENERIC STATUS OF THE SOUTHERN HEMISPHERE GENTIANS

BY W. R. PHILIPSON

Department of Botany, University of Canterbury, New Zealand

ABSTRACT

The two main accounts of the Gentianaceae (Bentham, 1876; Kusnezov, 1891) treat the genus *Gentiana* in a broad sense, despite many previous proposals to subdivide it into several genera. These divisions were not disregarded, but were treated by Kusnezov, where appropriate, as sections. At the present time there is no generally accepted definition of the genus *Gentiana* since recent floras take either a wide view of its limits (e.g., Grossgeim, 1952; Wiggins and Thomas, 1962; Gleason and Cronquist, 1963) or a more restricted view (e.g., Clapham et al., 1962; Burbidge and Grey, 1970). In the present account the status of the New Zealand and other southern-hemisphere species is considered in relation to the many groups found in the northern hemisphere. In South American literature (Fabris, 1955, 1958, 1959, 1960) and in Australian (Willis, 1957) the species have been treated in a segregate genus *Gentianella*, but in New Zealand literature (Allan, 1961) they have been retained in *Gentiana*, though Allan noted that their true position was open to question.

Kusnezov (1891) distributed the southern species between three of his sections. One of these, Dasystephana, has since been shown to be redundant (Fabris, 1958). The division between the other two sections based on the presence or absence of hairs within the corolla tube, appears artificial and unnecessary. The species of New Zealand, together with those from other southern regions, form a single group. The problem which still remains is the relationship of this group with the numerous sections found in the northern hemisphere. In the present paper the variation of some floral characters is considered within the sub-divisions of *Gentiana* and in some related genera. On the basis of these characters the classification of the group is reconsidered. Other lines of evidence such as ovary anatomy, phytochemistry, and cytology have relevance to this problem but sufficient evidence is not yet available.

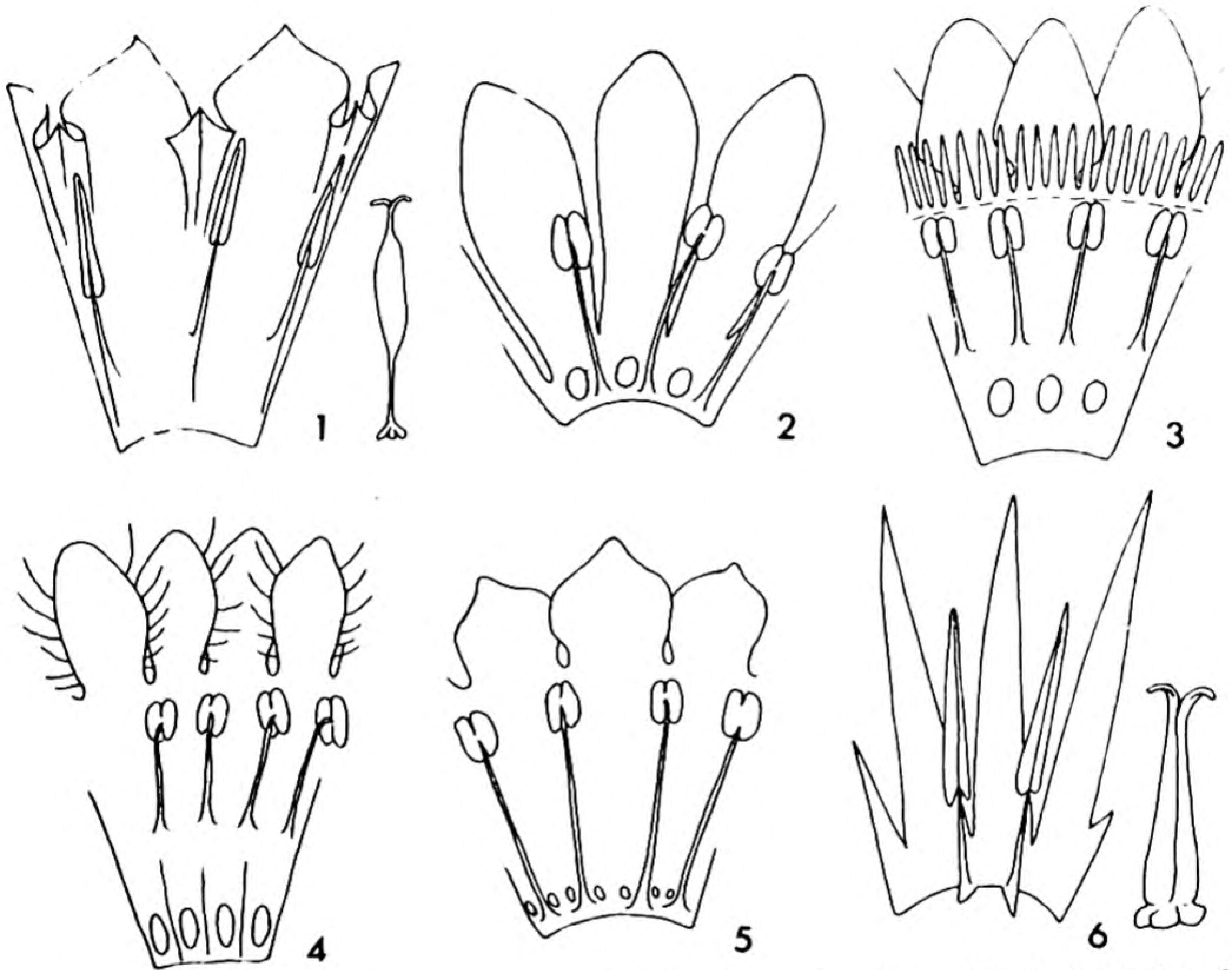
A highly characteristic feature of a typical gentian flower (Fig. 1) is the presence of *plicae* between the lobes of the corolla. Other features are the membranous sectors between the herbaceous lobes of the calyx, and the anthers fixed rather rigidly upright at the ends of the filaments. Other important characters are the nectary surrounding the base of the gynoecium which is narrowed into a hollow stalk. If a New Zealand gentian (Fig. 2) is compared with this flower it is evident that the corolla lobes are much more deeply divided and are not separated by *plicae*. Also the calyx lobes have no membranous sectors between them and the anthers are loosely attached. Finally the nectaries are located in small depressions on the inside of the short corolla tube and the ovary is not raised on a gynopodium. These differences result in two very distinct types of flowers. The contrasting features are linked with the functioning of the flowers. In the typical gentian flower the anthers are held stiffly around the ovary and shed their pollen into a central cavity: the stalk of the ovary then elongates so that the pollen is pushed to the open throat of the flower. Later the stigmatic arms diverge and become receptive. In contrast to this presentation mechanism, which is reminiscent of the Compositae, the New Zealand gentians rely on a movement of the stamens. At first these stand erect and when the pollen is exposed, the ovary is still small and the stigmatic arms closed together. The stamens later bend outwards and even protrude between the clefts of the petals. This position must ensure that no pollen can reach the stigmas of the same flower, which by this stage are fully receptive. The ageing stamens again move to the centre of the flower and so come to pollinate the stigma should it still be unvisited.

Flowers of this second type (Fig. 2) are not confined to New Zealand, including the sub-antarctic islands to the south of it, but occur in Tasmania and other parts of Australia, and are abundant in South America, extending northwards along the Andes to Colombia and Venezuela. Kusnezov divides the species with this flower-type into two sections: Andicola (in which the lower part of the filament and the corolla tube is tomentose) and Antarctophila (in which these parts are glabrous). Both these sections occur on both sides of the Pacific and their recognition separates very similar species. A truer concept of the southern gentians, therefore, is conveyed by treating them as a single entity. This is not to overlook that considerable adaptive radiation has occurred within the group and is shown by the vegetative diversity which is especially marked on the Andes. Nevertheless, the floral characters remain remarkably constant, even when adaptation affects floral biology, as in bird-pollinated species, in which the floral characters remain essentially unmodified except for the downwardly deflected flowers and the rigid pedicels.

Gentians with flowers like those in figure 1 occur across the three northern continents. In the old world they do not penetrate further south than the Highlands of New Guinea, but in the new world they have progressed along the Andes as far south as Argentina (Fabris, 1953). The northern half of the Andes, therefore, is the only region where the two flower-types occur together.

If the situation were as simple as this there would be no doubts of the usefulness of placing the two types in separate genera. There are, however, several other floral patterns within *Gentiana* in the wide and these must be discussed before conclusions can be reached.

Firstly, numerous gentians, spreading over the northern hemisphere have flowers resembling figure 3, and for convenience these will be referred to as the Amarella group. This flower-type lacks all the features of the typical gentian, but shows nearly all of those noted in the southern group. In addition, there is a conspicuous fringe of filaments at the throat of the corolla tube and the tube itself is relatively much longer than in the southern gentians. These two characters give the Amarella group a very distinctive appearance and this, combined with their separate geographical range, raises doubts about the closeness of their relationship to the southern group. It is true that Kusnezov reports the occurrence of two species of the Amarella group in South America, but this overlap of ranges is very slight. It should also be borne in mind that a few northern species with a fringe of processes in the corolla-throat have been segregated as a distinct section, section Comastoma, mainly on the grounds that the processes are vascularized in these species, whereas they are non-vascularized in Amarella.



FIGS. 1-6. Diagrams of opened flowers. Fig. 1. A typical gentian. Fig. 2. A member of the southern group. Fig. 3. A member of the "Amarella" group. Fig. 4. A member of the "Crossopetalum" group. Fig. 5. A member of the "Arctophila" group. Fig. 6. *Gentiana lutea*. Ovaries are included in Figs. 1 and 6 to show the basal nectaries. In the other figures the nectaries are shown as rounded areas near the base of the corolla.

Secondly, the species related to *G. ciliata* are so distinctive that we can regard them as another group of species. Several names have been applied to this group, often at the generic level, and for convenience they will be referred to here as the Crossopetalum group. Figure 4 is based on *G. paludosa*, and the following features are to be noted: the folds of tissue on the inner side of the calyx and the associated glands; the presence of small *plicae* between the corolla lobes (these are not present or very reduced in several species of this group); the large stigmas; the gynopodium; the ciliate margins of the corolla lobes; and the nectaries located within the corolla tube. About twelve species, showing combinations of all or most of the characters, together with other non-floral characters, occur in Asia, Europe and North and Central America. This well defined group shows no approach to the southern type.

A third group from the northern hemisphere comes very close in floral structure to the southern type of flower. We may use Kusnezov's name Arctophila for this assemblage of about a dozen species which are spread sparsely over North America and Asia. It may well be that these widely dispersed species do not form a natural group, since they vary in such features as the number of nectaries in the corolla tube, and some (for example *G. aurea*) have a calyx with somewhat membranous zones between the lobes. However, they agree at least in the negative features of the absence of the ringed throat characteristic of Amarella and of all the characters that distinguish Crossopetalum. I include *G. moorcroftiana* in this group (Fig. 5) although Kusnezov placed this species in Amarella. It is clearly of importance that one North American species *G. wislezeni*, may have flowers with or without filaments in the throat of the corolla tube and may, therefore, constitute a link between the groups Amarella and Arctophila, although other peculiarities (e.g., of the calyx) suggest that it is a divergent species. Clearly the flowers of Arctophila approach those of the southern type more closely than do any so far considered. However, they do not match them completely. All have a distinctly longer corolla tube, and many, though not all, have ten instead of the five nectaries found in all southern species. This, taken in conjunction with the facts of distribution, makes it doubtful whether there is any close relationship between these similar northern and southern groups.

Fourthly, a very distinctive European species, *G. lutea*, (which is indeed the original enzian of Central Europe) must be considered (Fig. 6). In this tall species with yellow flowers, the nectar is secreted at the base of the ovary, the corolla is divided almost to the base into narrow lobes which are not separated by *plicae* and the calyx is markedly assymetrical. The species is so distinctive that few would hesitate to place it in a genus of its own were it not for species which link it with the more typical flower-type. These are *G. punctata*, *G. purpurea*, and *G. pannonica*, which resemble *G. lutea* in habit, and in their assymetrical calyx, but which possess *plicae* between their corolla lobes. The occurrence of natural hybrids between *G. lutea* and these species is cofirmation of their close relationship.

Lastly, the full range of floral types within the *Gentiana* complex would have to include the three monotypic sections recognized by Kusnezov from the Himalayan-Chinese region, namely Imaicola, Stylophora and Megacodon, but since these diverge sharply from the southern type of flower, they need not be considerred here.

Attempts to classify the groups which have been informally described in the above account could take three broad courses, namely (i) to lump them all together treating *Gentiana* in the broadest sense; (ii) to regard most of the groups as separate genera; and (iii) to divide the group into two genera on the basis of the position of the nectary.

As we have seen both Bentham (1876)

and Kusnezov (1891) adopted the first course. They were probably influenced to take this decision largely because *G. lutea* combines the absence of *plicae* with the ovarian nectary, and possibly also by the somewhat intermediate character of the Crossopetalum group. The sequence of flower form from *G. punctata* and *G. purpurea* to *G. lutea* appears best explained as a secondary loss of *plicae* by *G. lutea*. The difficulties presented by Crossopetalum may best be solved by its separation as a genus (see below), but in any event the separation of the true gentians from all species with corolline nectaries appears justifiable. The problem would then remain whether all the remaining groups (Amarella, Crossopetalum Comostomum, Arctophilla, Megacodon, etc. and the southern gentians) are best regarded as a single genus (for which the name *Gentianella* is available) or whether some or all should assume (or in some cases resume) generic rank. Most modern authors have treated all these groups under *Gentianella* (e.g., Gillet, 1957; Fabris, 1955-60; Willis, 1957). This course has the disadvantage that the geographically and structurally distinctive uniform, and natural group, which is here referred to as the southern gentians, remains merged in a complex northern genus. The alternative of recognizing genera within species with corolline nectaries has two disadvantages, namely, that a considerable number of genera suggest themselves, and that the distinction between the southern group and Arctophila is difficult to define and may indeed be unnatural. An acceptable compromise would be to regard all but the most distinctive group i.e., Crossopetalum, as congeneric. In that event three genera would be recognized, namely *Gentiana*, *Gentianella*, and *Crossopetalum*.

The present account is intended to draw attention to some of the issues involved. Since other lines of evidence, such as placental anatomy, phytochemistry and cytotaxonomy will eventually help in the solution of this problem, it would be premature to propose new combinations under *Gentianella* for all the New Zealand gentians, though this has been done for most species from the southern regions. Also other genera of the Gentianinae with corolline nectaries must be taken into account in any global consideration of the position of the southern gentians. *Lomatogonium* is very similar to the New Zealand gentians, but the nectaries which are borne on the corolla near the insertion of the filaments are covered by a small flap of tissue. This character links *Lomatogonium* to the larger genus, *Swertia*, in which one or two nectaries are borne on the lobes or tube of the corolla and are surrounded by a fringed margin or by a flap of tissue. However, some species of *Swertia* are quite devoid of this margin or flap. For example, on Kilimanjaro I collected two species of *Swertia*: one *S. crassiuscula*, had the fringed nectaries typical of the genus, while the other *S. volkensii*, had not. Since the whole appearence of this last species was reminiscent of the New Zealand group of species it is at least possible that the southern gentians are represented on the mountains of equitorial Africa as well as the other southern continents.

REFERENCES

ALLAN, H. H. 1961. *Flora of New Zealand.* Vol. I. Wellington.

BENTHAM, G. 1876. In Bentham, G. and Hooker, J. D. *Genera Plantarum.* Vol. II. London. 799-820.

BURBIDGE, N. T., AND GRAY, M. 1970. *Flora of the A.C.T. Canberra.*

CLAPHAM, A. R., T. G. TUTIN, AND E. F. WARBURG. 1962. *Flora of the British Isles.* Cambridge.

FABRIS, H. A. 1953. Sinopsis preliminar de las Gencianaceas Argentinas. *Boln. Soc. Argent. Bot.* **4**: 233-259.

———. 1955. Nuevas especies de "*Gentianella*" del Peru. *Boln. Soc. argent. Bot.* **6**: 45-50.

———. 1958. Notas sobre *Gentianella* del Peru. *Boln. Soc. argent. Bot.* **7**: 86-93.

———. 1959. Sobre la identidad de dos especies Sudamericanas de *Gentianella. Boln. Soc. argent. Bot.* **8**: 24-25.

———. 1960. El Genero *Gentianella* en Ecuador. *Boln. Soc. argent. Bot.* **8**: 160-193

GILLETT, J. M. 1957. A Revision of the North American species of *Gentianella* Moench. *Ann. Mo. bot. Gdn.* **44**: 195-269

GLEASON, H. A., AND A. CRONQUIST, 1963. *Manual of the Vascular Plants of the North Eastern United States and Adjacent Canada.* Princeton.

GROSSGEIM, A. A. 1952. Gentianaceae. In *Flora of the U.S,S.R.* Shishkin B. K. and E. G. Bobrov, (eds). Vol. **18**. Leningrad. (English translation, Jerusalem, 1967.)

KUSNEZOV, N. 1891, ex E. GILG. IN A. ENGLER, AND K. PRANTL, *Die natürlichen Pflanzenfamilien* 4 (i): 80-86. Leipzig.

WIGGINS, I. L., AND J. H. THOMAS. 1962. *A Flora of the Alaskan Arctic Slope.* Toronto

WILLIS, J. H. 1957. Vascular Flora of Victoria and South Australia. *Vict. Nat.* **73**: 188-202.

Adv. Pl. Morph. 1972 : 423–428

SILVA AD SILENOLOGIAM 4. *SILENE TURBINATIGEMMA* ET *S. PURII* BOCQUET ET SAXENA, SPEC. NOVAE *CARYOPHYLLACEARUM*

BY G. BOCQUET ET N. P. SAXENA[1]

Institut f. spezielle Botanik, Eidg. technische Hochschule, Zürich, Switzerland

ABSTRACT

Two new species of *Silene* (*Caryophyllaceae*) are described and illustrated: *Silene turbinatigemma* and *S. purii* Bocquet et Saxena. These two species are taxonomically close to the section *Physolychnis* (Bentham) Bocquet, recently revised by the senior author (Bocquet, 1969). Nevertheless, they have been excluded from this section on account of certain morphological characteristics: the number of styles (3 to 5 instead of 5 in *Physolychnis*) and a racemose inflorescene in *S. turbinatigemma*. It is not possible at present to place *S. purii* in a particular section of *Silene*, until more abundant material is available. On the contrary, *S. turbinatigemma* is provisionally placed in the section *Graminifoliae*.

RESUME

Deux nouvelles espèces de *Silene* (*Caryophyllaceae*) sont décrites et illustrées ci-dessous : les *Silene turbinatigemma* et *S. purii* Bocquet et Saxena. Ces deux espèces sont proches de la section *Physolychnis* (Bentham) Bocquet, révisée per l'un des auteurs (Bocquet, 1969). Elles n'ont toutefois pas été retenues dans cette section en raison de certaines de leurs caractéristiques morphologiques : le nombre des styles (3 à 5, au lieu de 5 chez les *Physolychnis*) et, pour le *Silene turbinatigemma*, l'inflorescence racémeuse. Il n'est pas possible pour l'instant d'attribuer le *Silene purii* à une section connue du genre *Silene* : un matériel plus riche est nécessaire. Le *S turbinatigemma* par contre trouve au moins provisoirement sa place dans la section *Graminifoliae* Chowdhuri.

Silene turbinatigemma Bocquet et Saxena spec. nova

Silene foliis angustis, graminiformibus, scapis erectis, inflorescentia racemosa redacta, gemmis turbinatis, sect. *Graminifoliis* pertinens, tamen habitu Silenes chodatii non dissimilis.

Chamaephyton perenne laxe caespitosum. *Radix* ignota. *Caudex* lignosus, ramosus, ramis brunneis, subterraneis, elongatis, gemmas et radices adventivas fibrillosas apud internodia ferentibus, soboles numerosos emittentibus, qui rosulas steriles et scapos fertiles gerunt, imma basi vestigiis foliorum annorum praecedentium distincte contectos. *Scapi* adscendentes vel erecti, robustiores, simplices, (15-) 17-27 (-30) cm alti, flavi- vel brunneivirides (statu sicco), glutinosi,

---

[1] Present Address : Botany Department, D. N. College, Meerut (India)

pubescentiores, apicem versus pilosi pilis arctis, internodiis elongatioribus, 1-2 basilarium et 1-2 (—3) caulinorum foliorum paria gerentes; pili pluricellulares, albidi (statu sicco ! in vivo tamen verisimiliter± violaceitincti), 0·1—0·6 mm alti, sine glandulis terminalibus, tamen tota ab parte glutinosi, hirti vel hirtipilosi, scapi apicem versus quasi lanati et arctissimi. *Folia* inferiora subrosulantia, angustissime elliptica. 4-6 x 50-70 mm magna (lat. / long. ca 1:15); caulina angustissime ovata, gradatim redacta; omnia acuta, flaviviridia (statu sicco) interdum apicem versus nigrescentia, crassiora (id est in herbario satis corrugata), univervia, nervo supra indistincto, subtus prominente, superiore in pagina et praesertim secundum margines hirta, subtus praeter nervum fere glabra, marginibus revolutis, tamen basin versus complanatis et fere lanatis. *Inflorescentia* 5-7 flora, racemiformis; bracteae foliis caulinis similes, sed redactae. *Florum* alabastri turbinati, sessiles, obliqui vel fere horizontales, arcte hirsutipilosi; flores pedicellati, pedicellis (0·5) 1-2·5 (-3) cm longi, cernui vel nutantes. *Calyx* perinflatus, magnus, 11 mm latus, 13mm altus, subglobosus, vesiculiformis, in basi leviter umbilicatus, per maturitatem haud crescens, luteiviridis, limbo membranaceo arcte hirsuto, pilis similibus atque in scapo; nervi nigrescentes, conspicui, arctissime hirsuti, in dentibus haud conjuncti, parum ramosi; dentes traingulares, 3x3 mm magni, subacuti, marginibus ciliatilanatis. *Gonophorum* ca. 2 mm altum, 1·5 mm crassum, apicem versus lanatum, pilis albidis arctis. *Petala* calycem 7-8 mm superantia; unguis cuneiformis, media in parte, 1·5 mm latus, ca. 9 mm altus, superne in auriculas dilatatus et tum 6 mm latus, basin versus pilosus; limbus bifidus, lobis inaequaliter lobatis vel fissis, 6 mm latus, 7 mm altus intus sordide purpureus, extus viriditinctus, appendicibus binis, inaequaliter lobulatis, fornicatis, 3 mm altis, 1·5-2 mm latis in fauce praeditus. *Stamina* 10. *Ovarium* viride, in anthesi ca. 4x8 mm magnum. *Styli* 3-5. *Capsula* et *semina* ignota.

Type. — *Ludlow. Sherriff & Taylor* 5997 (holo BM).

Icones. — ce travail, tab. 1.

Etymologie. — de *turbinatus,* en forme de toupie et de *gemma,* pour bouton floral: allusion à la forme caractéristique des boutons floraux.

Habitat. — étage alpin, vers 4000 m, vires rocheuses et herbeuses, fentes de rochers.

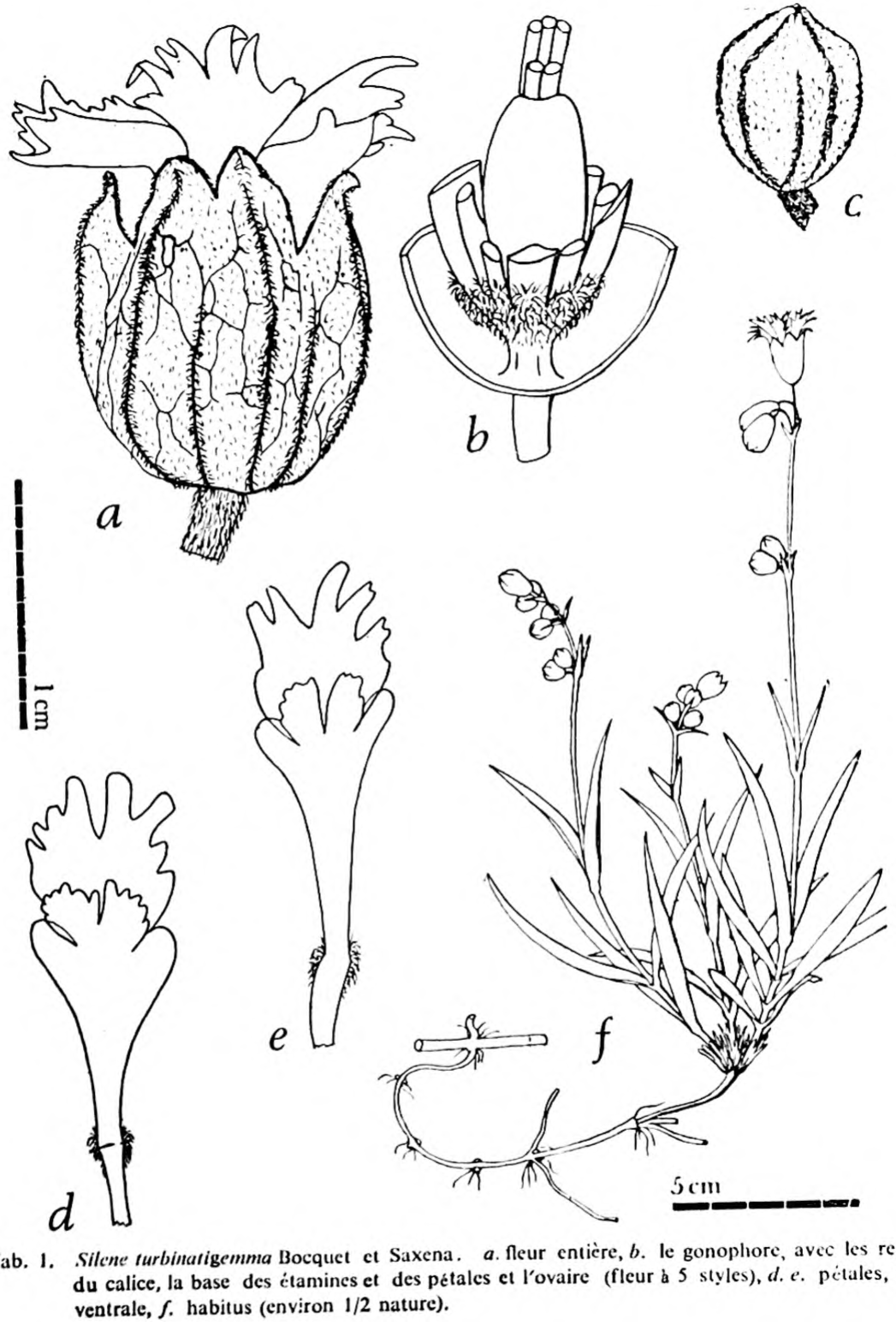
Echantillons connus. — Tibet, province do Kongbo, vallée du Tsangpo, vallée au dessus de Tse, alt. 3850 m, 29° 23′ N, 94° 22′ E, 2.8.1938, dans des crevasses rocheuses, pétales pourpre sale, extmt. verdâtres, *F. Ludlow, G. Sherriff & G. Taylor* 5479 (BM); id. Kulu Phu Chu, près de Paka, alt. 4200m, 29° 15′ N, 94° 25′ E, 27.7.1938, sur les vires herbeuses et découvertes de falaises rocheuses, *Ludlow, Sherriff & Taylor* 5957 (holotypus BM); id. *Ludlow, Sherriff & Taylor* 5958 (BM).

Distribution. — Endémique, du sud-est du Tibet, province du Kongbo, dans l'est de la vallée du Tsangpo.

Morphologie variabilite. — La description correspond étroitement au type. Il est difficile, vu le petit monbre des exemplaire connus, de décrire la variablilité de l'espèce. Cependant, les échantillons des biotopes les plus exposés (fissures des rochers) ont des feuilles plus étroites, plus, longues, plus graminiformes que ceux des vires plus ou moins herbeuses.

Le *S. turbinatigemma* est caractérisé per:

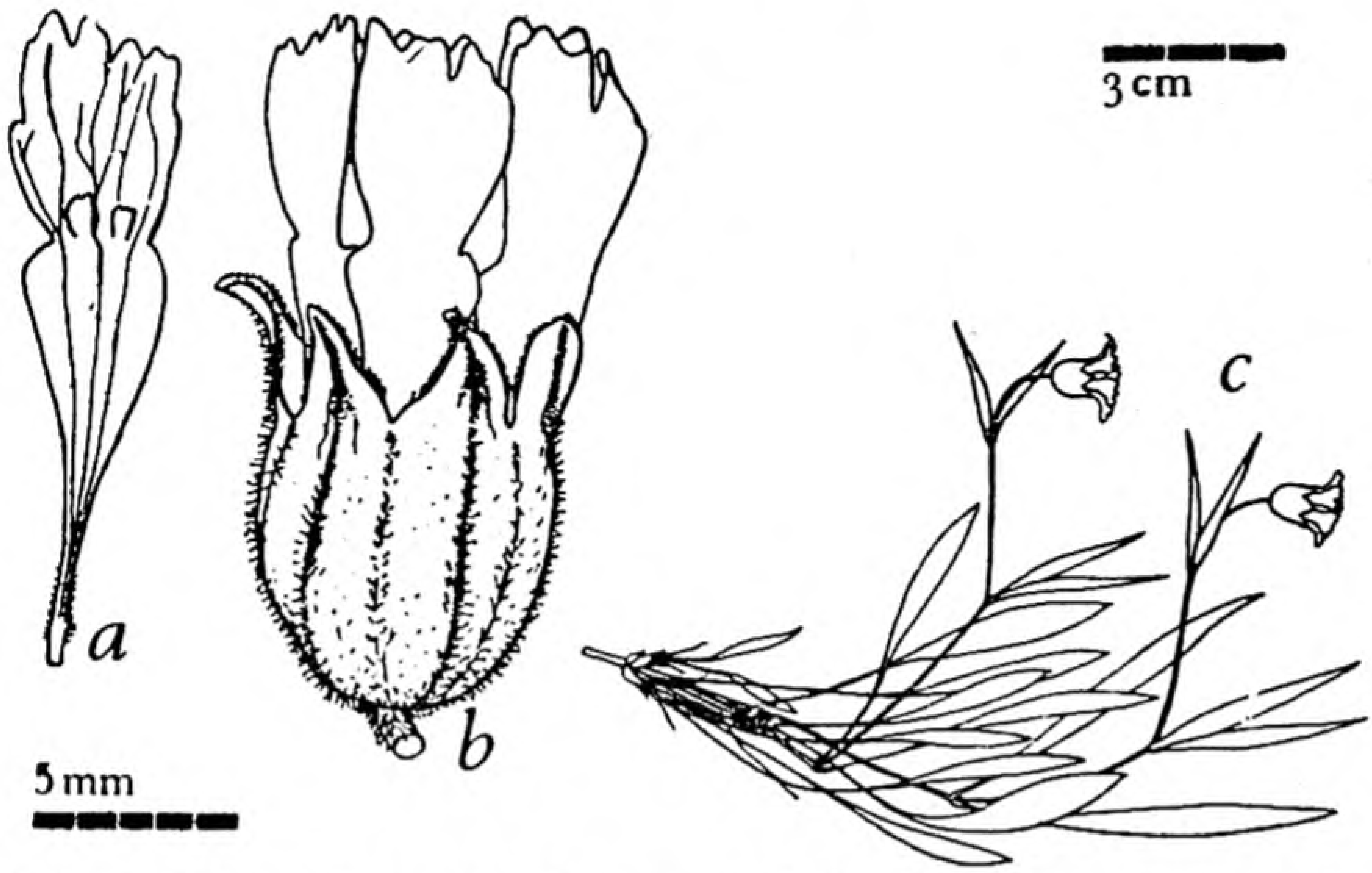
1. ses inflorescences reacémeuses



Tab. 1. *Silene turbinatigemma* Bocquet et Saxena. *a.* fleur entière, *b.* le gonophore, avec les restes du calice, la base des étamines et des pétales et l'ovaire (fleur à 5 styles), *d. e.* pétales, vue ventrale, *f.* habitus (environ 1/2 nature).

pauciflores a fleurs grandes;

2. par la forme turbinée de ses boutons floraux; renflés on sphère, ils sont surmontés par la pyramide de dents duc calice, appliquées comme des valvules les unes contre les autres.



Tab. 2. *Silene purii* Bocquet et Saxena. *a*. pétale, vue ventrale, *b*. fleur entière, *c*. habitus (environ 1/2 nature).

L'inflorescence correspond à l'une des formes de réduction possible du type général chez les silènes: le thyrse (*dichasiopanicula*). Il ne s'agit donc que d'une fausse grappe, d'où notre qualification "racemiformis". Du thyrse ne subsiste que la fleur terminale du rachis et les fleurs terminales de quatre inflorescences partielles sur deux entre-noeuds.

Ecologie. — Il s'agit d'un chaméphyte à souche souterranine grêle, mais très ligneuse, bien développée et ramifiée, porteuses de nombreux bourgeons souterrains, traçante. Le *S. turbinatigemma* se rencontre comme semi-chasmophyte sur des vires rocailleuses det herbeuses, où il gazonne lâchement. Il peut aussi se présenter comme chasmophyte vrai.

Le port bas, les feuilles épaisses, la présence assez abondante d'anthocyanes, les calices vésiculeux témoignent de l'adaptation aux conditions alpines: d'où la convergence, sinon la parenté avec la sect. *Physolychnis*.

Affinites. — Le *Silene turbinatigemma* rappelle par son aspect général:

(a) le *Silene chodatti* (sect. *Physolychnis* ssect. *Nigrescentes* Bocquet). Mais ses fleurs comptent 3-5 styles, son inflorescence est un racème pauciflore et ses boutons sont turbinés. Le *Silene chodatii* possède des calices infudibuliformes et non globuleux comme ceux du *S. turbinatigemma*.

(b) Certaines espèces américaines de la section *Graminifoliae* Chowdhuri: on retrouve les feuilles étroites, graminiformes l'inflorescence racémeuses, les calices enflés (à un degré moindre cependant); citons les *S. macounii* Watson, *S. parryi* (Watson) Hitchcock & Maguire, *S. scouleri* Hooker et *S. douglasii* Hooker.

(c) Enfin le *Silene chamarensis* Turcz., un membre asiatique de cette section

Graminifoliae. L'inflorescence est semblable, un racème, mais les fleurs du *S. chamarensis* sont nattement plus petites et d'un type plus "steppique", avec des calices plus coriaces que ceux du *S. turbinatigemma*.

Ces relations réticulées entre les sections sont caractéristiques des difficultés que l'on rencontre dans le genere *Silene:* le travail de Chowdhuri ne représente d'ailleurs qu'un premier pas vers une classification subgénérique des silènes. Cet essai est très utile, mais devrait être repris non plus "d'en haut", mais à partier des espèces.

Il s'ensuit que l'attribution définitive du *S. turbinatigemma* à une section du genera *Silene* ne sera possible qu'après une révision des espèces asiatiques du genra. Provisoirement, nous proposons la section *Graminifoliae,* en raison des inflorescences recémeuses et du nombre des styles. La parenté avec les *Physolychnis* est cependent à retenir: onytrouve également des inflorescences reacémeuses dans la ssect. *songaricae* (*S. maheshwarii* Bocquet, *S. drummondii* Hook., etc.).

Silene Purii Bocquet et Saxena spec. nova.

Silene chasmophytica, humilis, umbrosa, in fissuris rupium pulvinos laxos formans, scapis fesse adscendentibus, foliis fere glabris, tenuibus, angiustissime ellipticis, floribus solitariis, petalis conspicuis, *Silene falconeri* non dissimilis sed stylis 3 praedita.

Chasmophyton perenne humile, pulvinos laxos ad fissuras rupium formans. *Radix* ignota. *Caudex* lignosus, ramosus, ramis brunneis, in fissuris abstrusis, elongatis, gemmas et radices adventivas fibrillosas apud internodia ferentibus, soboles numberosos emittentibus, qui rosulas steriles et scapos fertiles gerunt, imma basi vestigiis foliorum annorum praecedentium laxe contectos. *Scapi* primum decumbentes, deinde fesse adscendentes, graciles, simplices, 5 -11 cm alti, humiles, in sicco flavivirides, glutinosi, pubescentiores, 1-2 basilarium, 2 caulinorum foliorum paria gerentes, cum 2 internodiis parum elongatis; pili pluricellulares, albidi (statu sicco! in vivo tamen ±violaceitincti) saepe cum glandula terminali, hirti, patentes, 0·2-0·4 mm alti. *Folia* basilaria subrosulantia, angustissime elliptica, 4-6 x 40-60 mm mgna (lat./long. ca. 1:12), in petiolum gracile, 3-4 cm longum attenuate; caulina similia sed gradatim redacta; omina acuta, in sicco flavoviridia, tenuiora, uninervia, nervo supra indistincto, subtus leviter prominente superiore in pagina hirtula, subtus et praeter nervum glabra, marginibus ciliatis. *Inflorescentia* uniflora, bracteis binis foliorum similibus, tamen redactis. *Flos* nutans, pedicellis arcte glandulosihirsutis, 1-4 cm longis. *Calyx* subinflatus, mediocris, 7 mm latus et 10 mm longus, campanulatus, ore aperto, in basi leviter umbilicatus, per maturitatem haud crescens, luteiviridis, limbo membranaceo, subglabro; nervi 10, inconspicui, arcte hirsuti, in dentibus haud conjuncti, simplices; dentes triangulares, ca 3 x 3 mm magni , subacuti, marginibus glandulosiciliatis *Gonophorum* ca 1·5 mm altum, 0·5 mm crassum, glabrum. *Petala* calycem ca 6 mm superantia; unguis cuneiformis, media in altitudine 1 mm latus, 1 mm supra calycem exsertus, 10 mm altus, basin versus ciliatus, superne in auriculas discretas, ca. 0·5 mm latas in utroque latere dilatatus; limbus bilobatus, lobis inaequaliter lobulatis, 4-5 mm latus, ca 7 mm altus, dentibus binis exterioribus indistinctis praeditus verisimiliter sordide purpureus, appendicibus binis, ca 0·5-0·7 mm latis et 0·6-1 mm altis , truncatis et inaequaliter crenulatis, fornicatis in fauce munitus. *Stamina* 10, os aequantia. *Ovarium* viride, in anthesi ca 4x8 mm magnum. *Styli* 3. *Capsula* et *semina* ignota.

Type. — *Cooper* 3227 (holo E).

Icones. — ce travial, tab. 2.

Etymologie. — haec species cl. V. Puri dedico, meerutensi, rerum morphologicarum imperatori, magistro eximio et amico carissimo.

Habitat. — étage alpin, comme chasmophyte, dans des fissures a l'ombre vers 4000 m.

Echantillon Connu. — Bhotan, Phajurdin Jimpu (?), 4200 m, 15. 8. 1914, "in shady rock crevice", *R. E. Cooper* 3227 (E, holo).

Distribution. — endémique, du Bhotan.

Ecologie. — comme le *Silene turbinatigemma,* le *S. Purii* possède une souche grèle, mais lingneuse et certainement très développée. Les rameaux de cette souche se glaissent le long des fissures. Cette structure de la souche se retrouve chez bien d'autres silènes de l'étage alpin: en Europe, on peut citer par exemple le *S. cordifolia.* Ces silènes sont en général capables de s'adapter également aux conditions de l'éboulis. On peut donc s'attendre à retrouver le *Silene purii* dans d'autres habitats.

Il est possible que l'échantillon type soit la forme d'ombre d'une espèce croissant habituellement dans des stations plus ensoleillées. Ces plantes devraient alors posséder des feuilles plus raides et graminiformes. Nous pensons toutefois que le *S. purii* est une bonne espèce endémique du Bhotan.

Affinities. — elles sont difficiles à établir en raison de la pauvreté du matériel et l'état précaire de nos connaissances sur les *Silene* centre-asiatiques.

Le *Silene purii* n'est pas sans rappeler par son apparence certaines des espèces de la sect. *Physolychnis* ssect. *Physolychnis:* par exemple les *S. falconeri* (Rohrb.) Bocquet et *S. gonosperma* (Rupr.) Bocquet ssp. *himalayensis* (Rohrb.) Bocquet (au moins dans le cas des échantillons particulièrement bas!). Il ne peut cependent appartenir à la section *Physolychnis* en raison de ses fleures à 3 styles.

C'est vraisemblablement à la sect. *Odontopetala* Schischikin ex Chowdhuri qu'il faudrait rattacher le *S. purii.* La sect. *Cordifoliae* pourrait aussi être prise en considération La délimitation de ces deux sections est d'ailleurs loin d'être claire[1].

---

[1] *Remerciements:* études rendues possibles par une subvention du Fonds national de la recherche scientifique suisse (requête 3. 7. 1968).

REFERENCES

BOCQUET, G. 1969. *Revisio Physolychnidum (Silene sect. Physolychnis).* Phanerogamarum monographiae. Cramer, Lehre.

CHOWDHURI, P. K. 1957. Studies in the genus Silene. *Notes Roy. Bot. Gard. Edinburgh* **22:** 221-278.

STEARN, W. T. 1966. *Botanical Latin* Nelson, London et Edinburgh.

Adv. Pl. Morph. 1972: 429–447

MORPHOGENESIS IN THE COMING DECADES*

BY S. C. MAHESHWARI

Department of Botany, University of Delhi, Delhi, India

Mr Chairman, Professor Puri and fellow botanists, I must tender an apology for not having been able to attend the earlier deliberations. Belated as my appearance here is, I do not want to lose any more time in acknowledging how very privileged I feel to have been invited to participate in this symposium. I wonder how many in the audience are aware that Professor Puri has known the speaker since childhood. We have had one common teacher and I know in how much esteem and affection he was held by his teacher. I wish it to be known that my generation loves him just as much and I humbly dedicate this contribution to him.

TO THE SUBJECT – WHY CHOSEN

It is a fashion these days to talk about the future—many futurist societies and magazines have come into existence in recent years. But I am not after the fashion; I chose to discuss the future not as an astrologer but more from the point of view of stock taking and planning for the future. The great and unprecedented problem in this century, arising out of rapid progress, is that it is often difficult to find our bearings—the changes come so fast that almost overnight one can be swept off one's feet and often hopelessly left far behind. It is particularly true of us scientists. It has thus become incumbent on each one of us to think, to understand and constantly assess whether we are on the right track and whether our ship is steering on the right course. For many reasons, my own mind has been very much preoccupied with the future of my own research, research in my own department, and even botany in the country, and I thought it would be most appropriate if I could talk about morphogenesis, the subject of my specific interest. As a morphologist, I can say that this is a topic that would be close to the hearts of many in the audience. The fact is that my own interests are due to the opportunity I have had in younger days to undergo classical botanical training and to leaf through such books as Goebel's Organographie der Pflanzen, Engler and Diels' Pflanzenreich, Haberlandt's Physiologische Pflanzenanatomic and others richly endowed with illustrations of the morphegenetic patterns in the plant world.

DEFINITION OF MORPHOGENESIS

I do not wish to spend too much time on the definition of morphogenesis. Indeed, there are as many definitions as authors. But the situation has its own charm. I do not know who invented the word, but certainly it is a delightfully flexible,

---

* This paper is based on a special lecture delivered by the author at the symposium on "The Biology of Land Plants" held at Meerut in June, 1972.

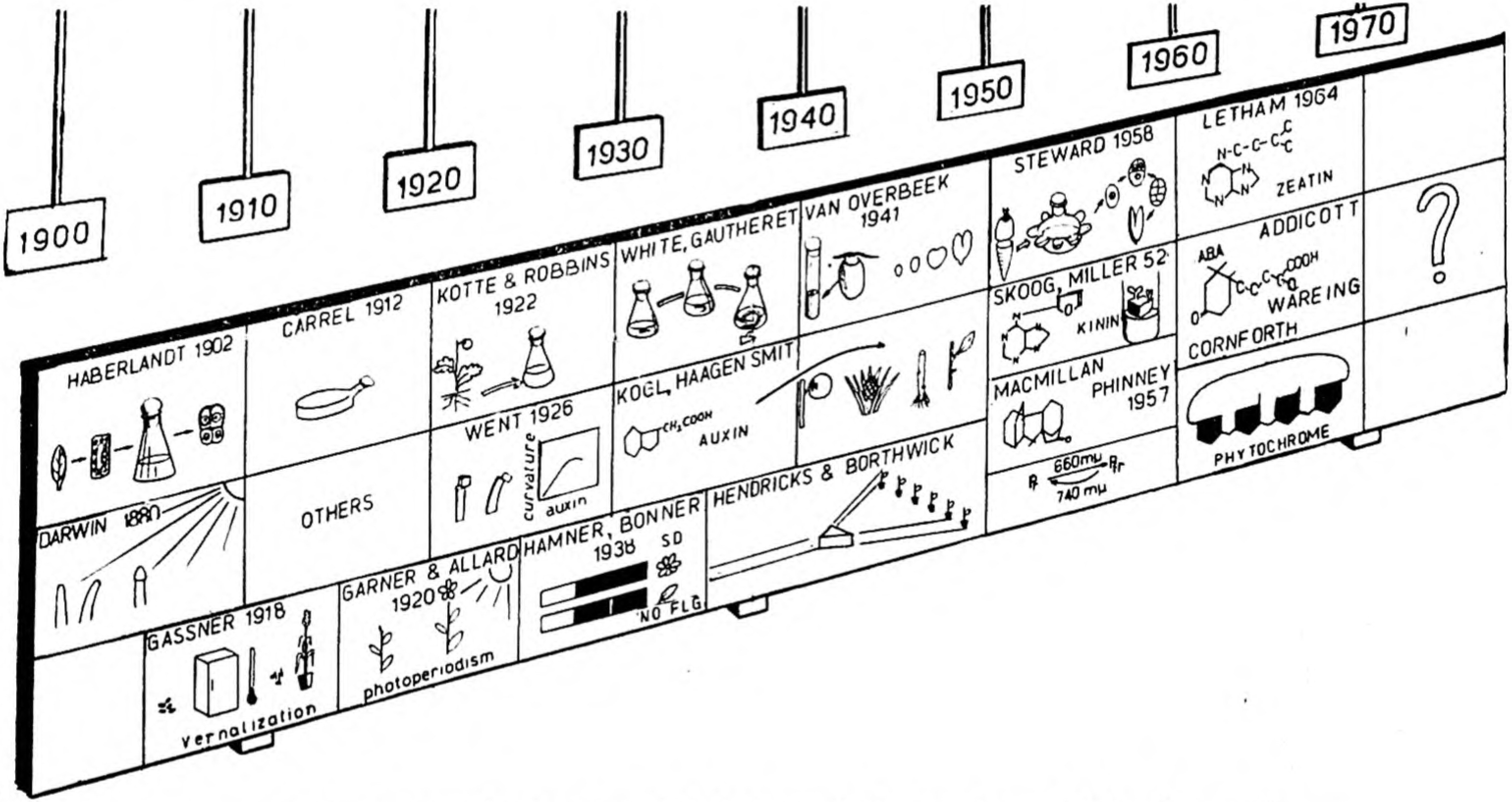

1900
1910
1920
1930
1940
1950
1960
1970
HABERLANDT 1902
CARREL 1912
KOTTE & ROBBINS 1922
WHITE, GAUTHERET
VAN OVERBEEK 1941
STEWARD 1958
LETHAM 1964
N-C-C-C
ZEATIN
ADDICOTT
ABA
COOH
WAREING
CORNFORTH
SKOOG, MILLER 52
KININ
MACMILLAN
PHINNEY 1957
WENT 1926
curvature
auxin
KOGL, HAAGEN SMIT
CH₂COOH
AUXIN
DARWIN 1880
OTHERS
HAMNER, BONNER 1938
SD
NO FLG
HENDRICKS & BORTHWICK
660mμ
740 mμ
PHYTOCHROME
GASSNER 1918
vernalization
GARNER & ALLARD 1920
photoperiodism
?

FIG. 1. A panoramic view of the principal advances in plant morphogenesis during the century.

all-embracing term and a theme on which every botanist can make a contribution. Morphogenesis is in fact edging out molecular biology. As a friend remarked in a lighter vein, by saying that our interests are in morphogenesis, one can pass off as a modern botanist or biologist, no matter how ignorant one may be! But to return to seriousness, morphogenesis is to my mind a term that is more or less synonymous with differentiation. To me both imply an enquiry into the causes—physical or chemical—that underlie ordered development.

THE PAST

Figure 1 gives a panoramic view of the great achievement of our predecessors during the past 70 years of the century. Many great and favourite names appear in it; examplewise, Haberlandt (1902) who first conceived the idea of cell and tissue culture. He attempted the culture of isolated palisade cells and manipulate them to change their morphogenesis at will though he did not succeed in this venture. Continuously growing cultures in synthetic medium were first achieved by P.R. White (1934) in the thirties. In the forties, Van Overbeek et al. (1941) introduced the now famous coconut milk, and employing this Steward (1959) in the fifties was able to score a spectacular success, that of demonstration of totipotency in plant cells. He showed that embryos could arise almost from any tissue. To be sure, totipotency of cells had been deduced from a great many natural observations, but for the sceptic this was a spectacular demonstration. The work proved once and for all that differentiation was not normally a result of chromosome or gene loss but due to ordered switching on and off of genes. Some colleagues would end the history of morphogenesis at this point. However, this in my opinion is grossly incorrect—there are other important concepts that have come in this period and which are of the utmost importance to morphogenesis. The discovery of growth hormones is one of these. It is Darwin's experiments around 1880 that set in motion the researches culminating in the discovery of auxin, the first plant growth hormone, isolated and identified later as indoleacetic acid by the dutch chemists Kögl et al. around 1934. The next hormone, discovered by Skoog and Miller in the early 1950s, is kinetin and this is an excellent though perhaps the only example of the use of tissue culture technique to isolate a new class of hormones. Kinetin, itself, is not a natural compound, but following this lead Letham (1963) in New Zealand isolated a few years ago the first naturally occurring cytokinin, zeatin, which too is a derivative of adenine. The important role of this class of hormones (called kinins) in cell division and in shoot and bud differentiation is too well-known to need emphasis. Another of the great achievements, by plain and simple growing of plants in pots, came through the work of MacMillan et al. (1961), Phinney et al. (1957) who isolated gibberellins in the higher plants and showed that they had profound developmental influences. Other important advances are the recognition of ethylene (Pratt and Goeschl, 1969), not shown in the diagram and abscisic acid as native regulators of plant growth. Everyone, I hope would appreciate the importance of inhibitors—were it not for abscisic acid or its allies (Addicott and Lyon, 1969; Wareing et al., 1968) every plant would have grown like the famed giant redwood trees or the bamboos! Important to morphogenesis are also the great discoveries in light regulation of plant growth. Blazing the trail set by Garner and Allard in 1920 who discovered the phenomenon of photoperiodism and Hamner and Bonner who in 1938 discovered that night interruption of an inductive dark period would completely abolish flowering, Hendricks and Borthwick in 1959 isolated phytochrome. With this, we close our brief survey of the history of morphogenesis of the past 60-70 years. I have probably omitted some favourite names but this is unavoidable if one must telescope 10 years in a minute.

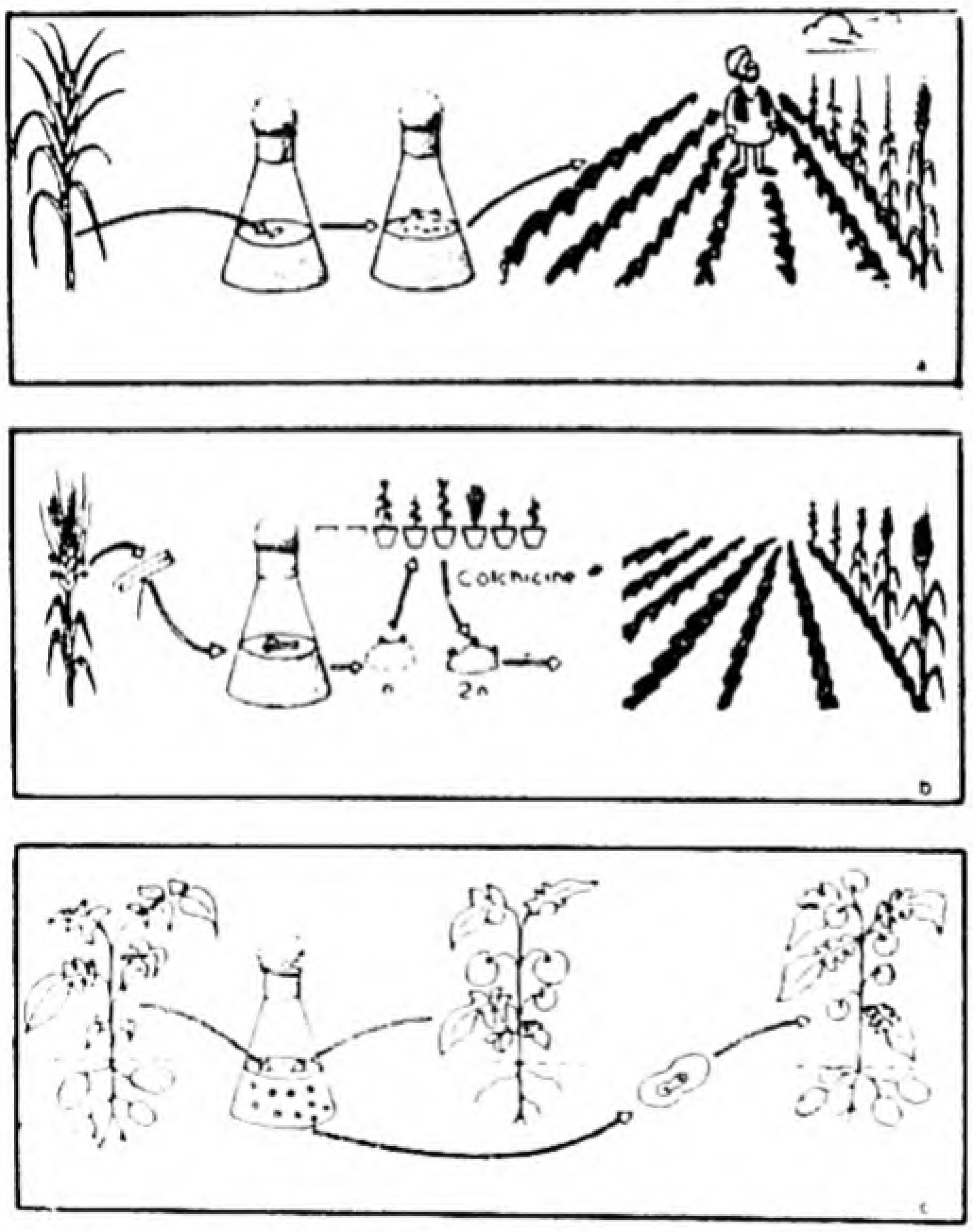


FIG. 2. Use of tissue culture in agriculture—some possibilities.

The sum and substance of all the work done so far is that plant growth and morphogenesis are caused by (a) a number of growth regulators and (b) physical agents like light and temperature, all of which in subtle ways—yet to be discovered—control plant behaviour (Fig. 2.)

WHAT OF THE FUTURE ?

We now ask the question as to how the study of morphogenesis will shape in the future years ? I would predict that in future it is likely there will be two broad and relatively independent directions of growth. Tissue culture may find much application in agriculture and horticulture though so far as fundamental botany is concerned, in my opinion, it would be only one of a dozen or more techniques important for the pursuit of this study. It is true that morphogenesis is often equated with tissue culture, but this is as absurd as trying to equate or study genetics by acetocarmine squashes or plant physiology by chromatography.

However, since mysteries of morphogenesis have often inspired work in tissue culture, in the first few minutes we shall study the potentiality of this technique in agriculture. The six frames of figures 2 and 3 represent some of the things that may be done in the next 20 years. It is conceivable that tissue culture may find widespread application in inducing adventive embryony in tissues of cultivated plants (Fig. 2a). There may of course be the usual difficulties encountered in tissue culture—namely, callussing of cells, chromosomal aberrations, and the like, but theoretically at least these difficulties may be overcome. In the coming decades this can be a great boon in sending clones and stock materials from one country to another. The second frame (Fig. 2b) depicts the raising of pure lines by the anther culture technique (Guha and Maheshwari, 1964). Pioneered by one of my former pupils and colleagues, Dr Sipra Mukherjee, this discovery attracted worldwide attention for its potential usefulness in raising of pure lines of selected genetic varieties. The implications of haploid plants are so very great that efforts are being made by European botanists to establish an exclusive international institute to work on this technique (Melchers, 1972). The third frame (Fig. 2c) shows the technique of isolating protoplasts by incorporating cellulase and pectinase in media and then fusing the naked cells in a suitable manner (Power et al., 1970). This technique, as visualized in the figure, may in the coming decades enable one to raise hybrid plants like the "pomato" with fruits of tomato and tubers of potato! There are many possibilities although one must state here that to date only cells have been fused, not their nuclei. In animal cells such as those of men and mice where such fusion has been reported, the chromosomes of one of the parents are gradually thrown out as the divisions continue. Probably in the next few years we shall know whether or not this technique has any future or not. If not, one can resort to the technique of in vitro fertilization pioneered by Kusum Kanta (see Kanta et al., 1962) under my father's guidance at the University of Delhi (Fig. 3a).

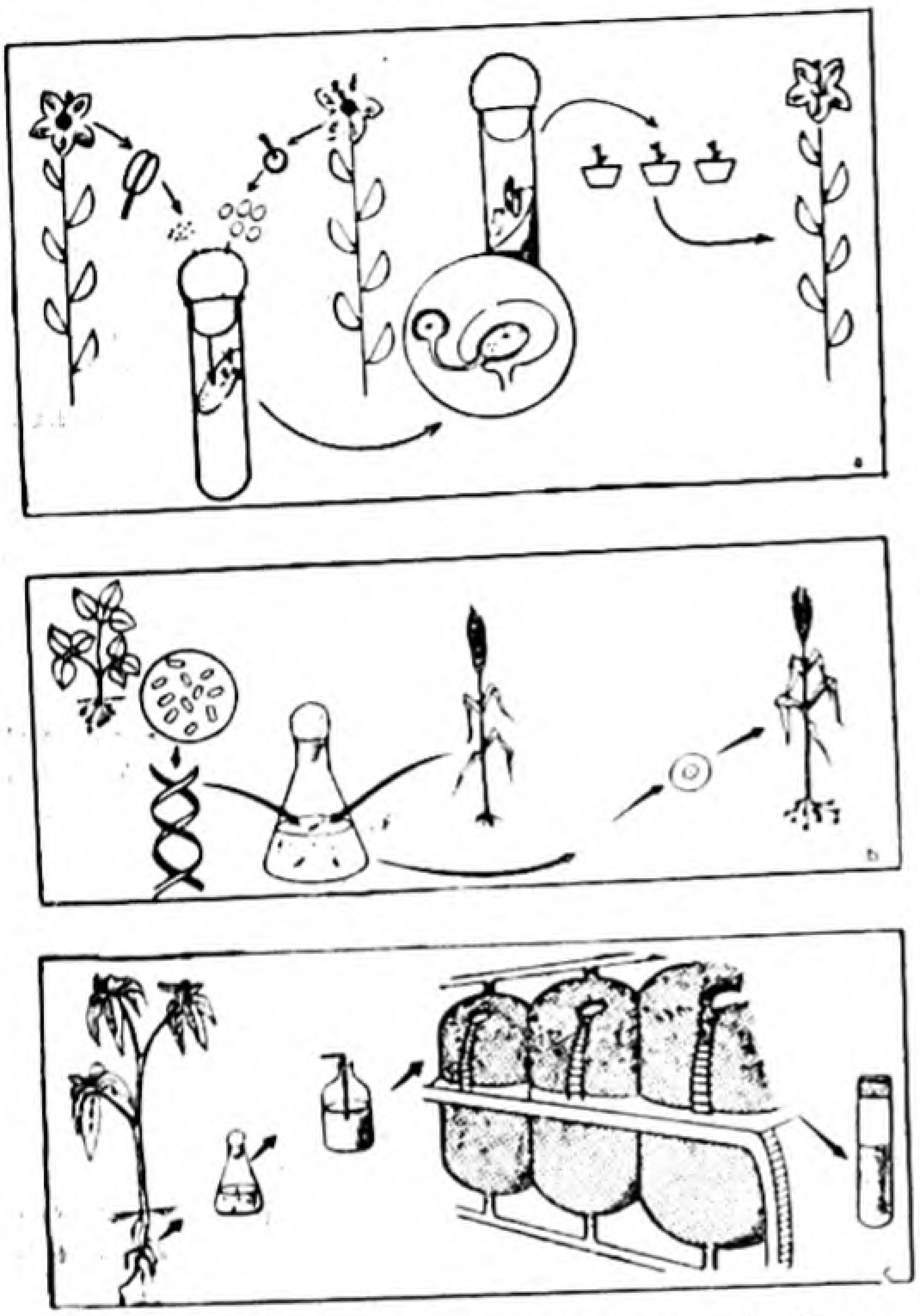


FIG. 3. Use of tissue culture in agriculture—further possibilities.

Tissue culture also has many possibilities in the growing art of genetic engineering. By the use of single cell suspensions in sterile culture, it might become possible to incorporate free DNA (extracted by biochemical procedures) from other tissues or even from bacteria. Thus it might be possible to raise plants with nitrogen fixing genes or with nodules (Fig. 3b). Finally, tissue culture has bright prospects in pharmacognosy—in making drugs such as reserpine from *Rauwolffia* by scaled-up large-volume cultures (Fig. 3c). Of course whatever possibilities I have indicated demand a great deal of painstaking work.

FUNDAMENTAL ASPECTS

But then we come back to our dear plant (Fig. 4). It is possible that applied research may yield some new facts of fundamental value. However, my personal estimate is that this approach, while relevant to agriculture, will not be the most rewarding for studying morphogenesis. In other words, I do not quite see how cultures of adventive embryos, fusion of protoplasts, or even culturing of anthers of newer plants to produce haploid embryos from pollen grains, will answer the great questions of morphogenesis like how and why hormones act in the way they do—questions such as why auxins promote rooting, why gibberellins bring about maleness or why in the long-day plants flowering is promoted by gibberellins, but in short day plants like *Lemna* by cytokinins.

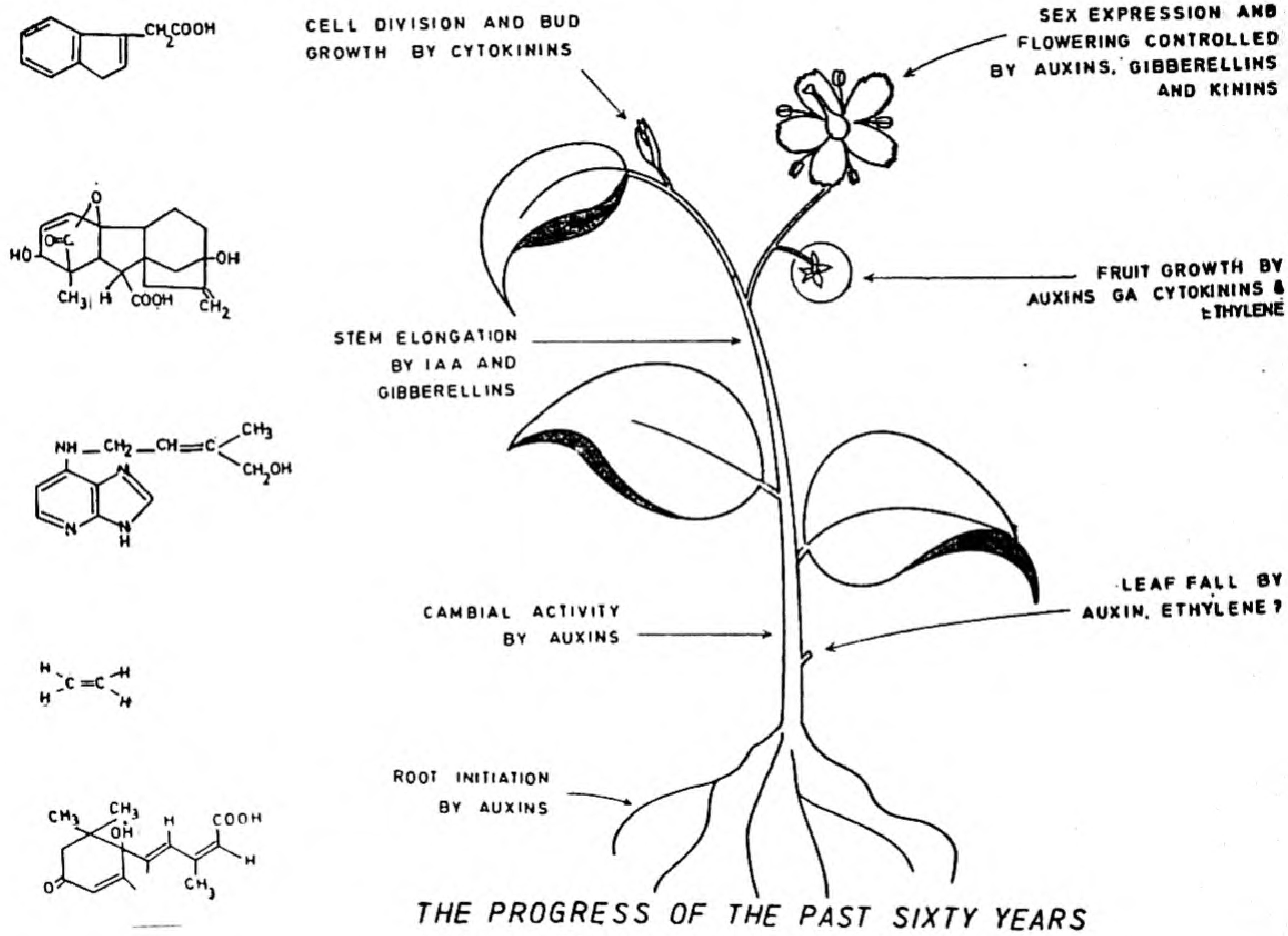
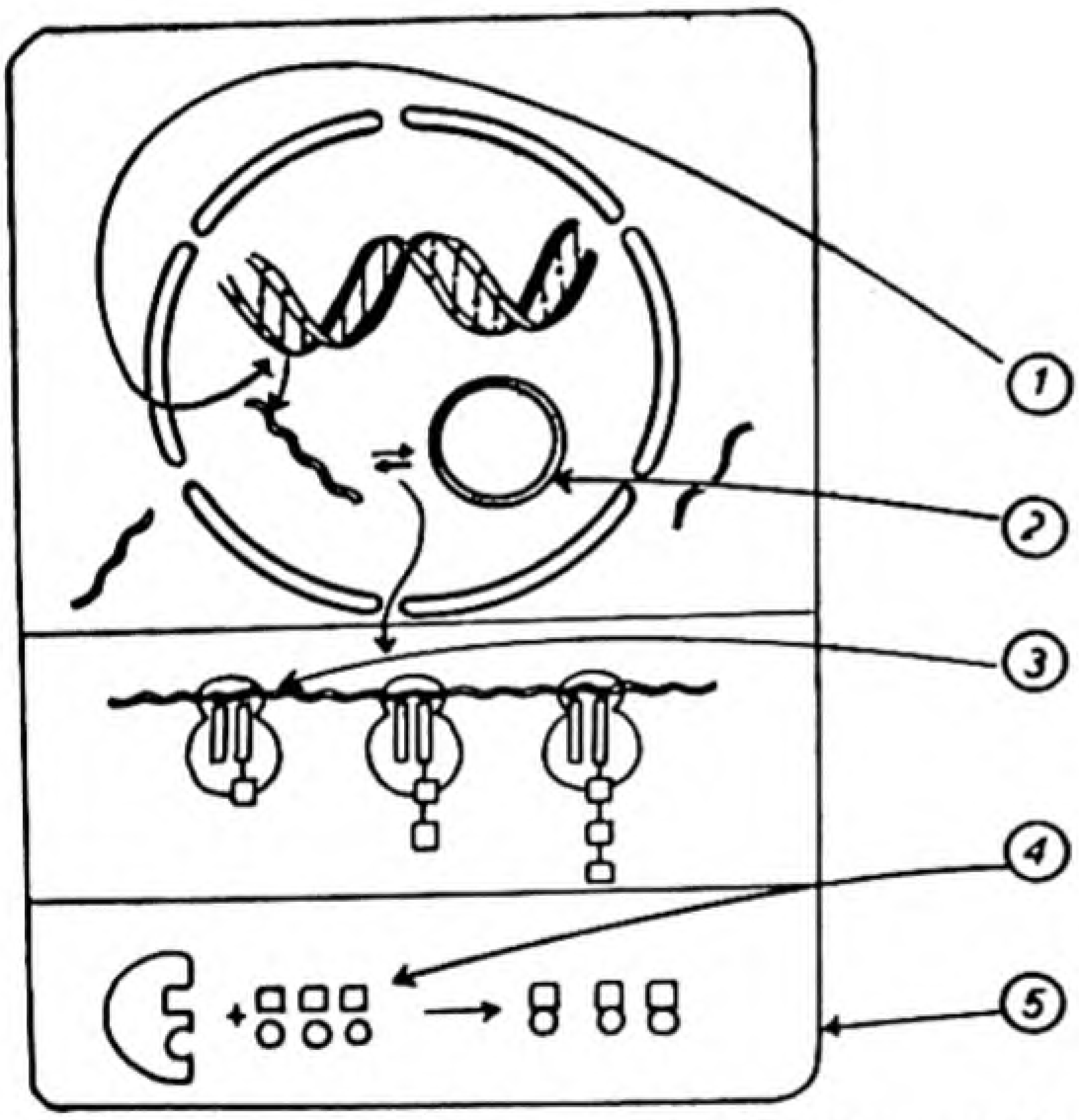

CELL DIVISION AND BUD GROWTH BY CYTOKININS
SEX EXPRESSION AND FLOWERING CONTROLLED BY AUXINS, GIBBERELLINS AND KININS
FRUIT GROWTH BY AUXINS GA CYTOKININS & ETHYLENE
STEM ELONGATION BY IAA AND GIBBERELLINS
LEAF FALL BY AUXIN, ETHYLENE ?
CAMBIAL ACTIVITY BY AUXINS
ROOT INITIATION BY AUXINS
THE PROGRESS OF THE PAST SIXTY YEARS

FIG. 4. The chemicals that control plant growth.

It seems to me that for answers to such questions as I have just raised—the kind of problems that need to be investigated in the universities as opposed to agricultural institutions—a completely different approach is needed and is being pursued. Tissue culture should and, may, have some role but not any more than any single technique has in a complicated investigation. The causes that underlie differentiation and morphogenesis—if we are serious—comprise molecular events and have to be sought at that level. My objective in inviting attention to figure 5 is not to show you the school book facts of how DNA makes mRNA and mRNA makes enzymes—it would be presumptuous for me to do that. But what I want to illustrate is where in cells control and regulation points are located and where decisions are made, say to make a flower or a leaf. In the cells, as the diagram depicts, there are five major points of control of overall general metabolism. The *first* major control operates in the nucleus—more precisely in chromosomes—where all the master plans for the development of an organism reside. As we shall see a little later a great deal of extraordinarily interesting work of immediate relevance to morphogenesis is going on to find how the genetic switch operates. A *second* control point, as is becoming apparent and which appears to be unique to the higher organisms, is that even after a messenger RNA has been made, a decision can be made whether or not it should work. As the diagram shows it can roll up and become quiescent. A *third* control point is at the level of protein synthesis—we shall see it is not that protein synthesis must necessarily follow if the messengers as well as ribosomes exist. In fact, there can be red and green signals. And *fourth,* we must find out how an enzyme activity is regulated (we know already that it is not necessary that enzymes must work even though they may be there). And finally, we must know how membranes work. The picture of membranes as merely passive inert structures is changing rapidly. On the contrary it seems they are under the direct and subtle control of hormones and of even light. There are many new and interesting concepts and ideas which have been proposed regarding each of these aspects which are going to occupy the attention of the foremost botanists and zoologists of the world in the next 20 years and in the remaining time I would like to discuss them.



WHAT MUST WE FIND TO FATHOM THE DEEPER MYSTERIES OF MORPHOGENESIS ?

① Know how genes are switched on and off.

② Know how a messenger RNA can become active or inactive

③ Know how ribosomes determine which message to translate and which not to.

④ Know how a cell controls activity of enzymes.

⑤ Know how membranes function.

FIG 5. Some tasks for the future.

As starting point, let us go back to the chromosomes and examine Fig. 6. As everyone knows, each cell of a higher plant or animal has about 3 feet of DNA (many millions of miles per individual). Through the work of Jacob and Monod (1961) we know how gene activity is controlled in bacteria (Fig. 6a). We know that, here, many related structural genes, i.e., genes that produce messengers or enzymes, have a common operator which, in turn, is under the control of a regulator gene through a proteinaceous repressor molecule. This is now elementary college level biology and I take it that most people are familiar with it. The repressor normally sits on the operator preventing the enzyme RNA polymerase from proceeding and transcribing the genes—but if a suitable small molecule is present the repressor is so altered that it does not any more do its job. Usually, these inducer molecules are substrates like lactose; lactose induces the *lac* operon to synthesize the enzymes β-galactosidase, permease and transacetylase all of which are required for the entry and utilization of lactose. The key point in the whole scheme is that the repressor molecule is a protein and which acts by *conformational* change from one shape into the other. The idea of conformational change is basic to the understanding of molecular mechanisms of morphogenesis and differentiation—the point is that the protein molecules are flexible like rubber or sponge. Combination with an external small molecule or metabolites can change the conformation of the repressor to another state so that it can no longer bind or if it were already bound it comes off the DNA. Such is the way the synthesis of many of the major enzymes is controlled. The inducer can be any small molecule and many kinds of repressors and inducers exist in bacteria.

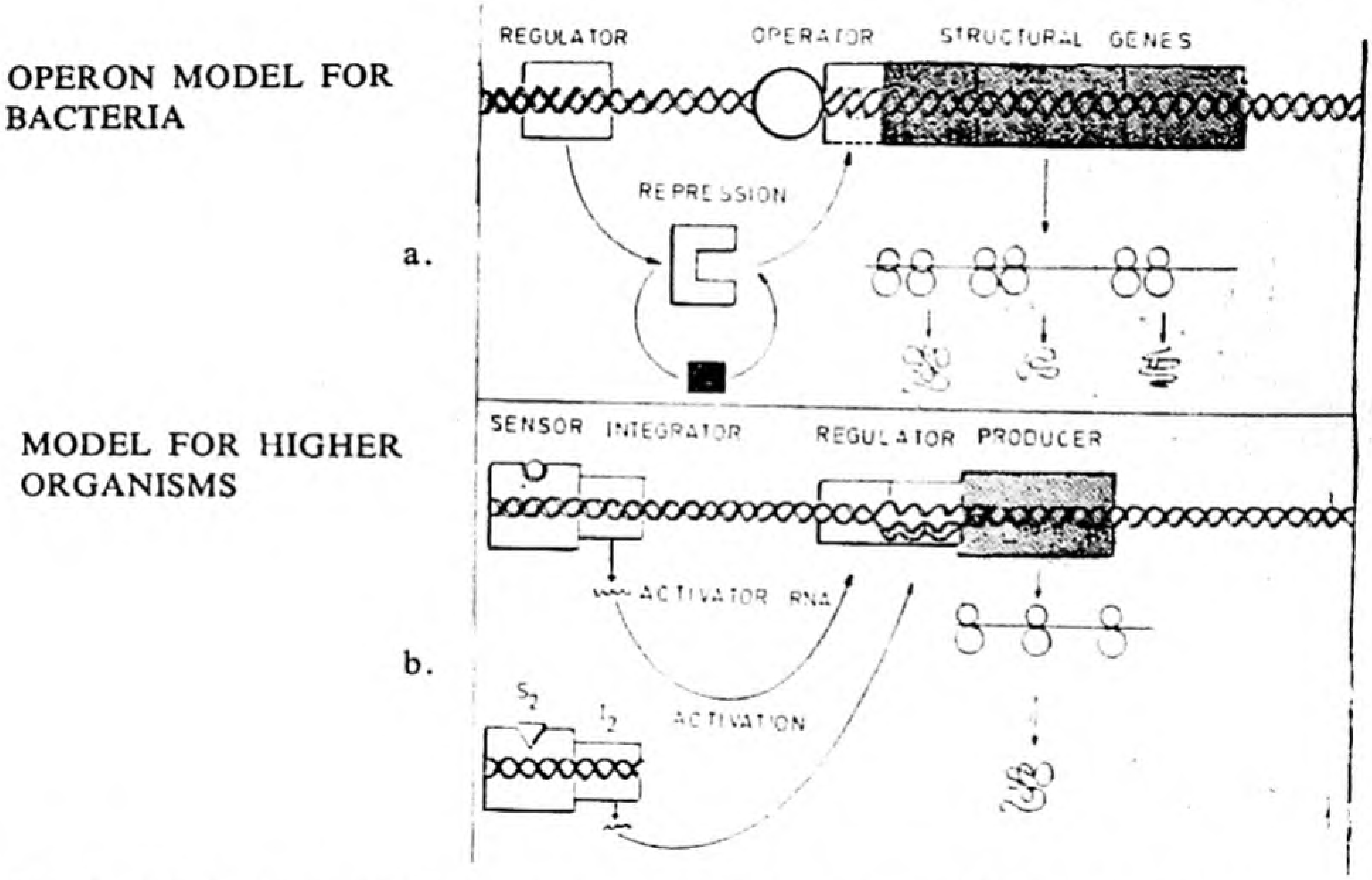


FIG. 6. Models for control of gene expression in the lower and higher organisms.

The lower part of the same figure (Fig. 6b) shows a model of regulation of gene activity proposed for the higher organisms (Britten and Davidson, 1969) —it is in some ways similar and in some ways dissimilar to that in bacteria. Most probably, the higher organisms *do not* have linked multiple-gene operons. However, it appears that the higher plants and animals including human, may have individual genes under a similar control. In the figure are *producer* gene —the terminology is a bit different, but the function of the producer gene is of course the same as the structural. "Producer" may indeed be a better term since such genes produce the messengers for enzyme synthesis. Contiguous to the producer gene is a leader sequence called *regulator* here. On the same chromosome or another there is also an *integrator* gene which controls this regulator so that the latter may function. The basic plan is similar to that in bacteria and I suggest we just do not worry about the changes in names. Now let us look at the points of distinction. One is that the control may not be through a protein but an RNA, christened as activator or chromosomal RNA (Sivolap and Bonner, 1971). There is also the point that the control here is positive and not negative—here, normally, all genes are repressed and you require an activator RNA to start production of messengers. (Let us not presently worry how the activator RNA activates—we shall come to that later). The second distinction, and a more important one, is that the integrator gene may be under the control of another contiguous gene labelled here as the *sensor* gene. There is, as is evident, a heirarchy of control. The third point, probably the most important in this model—which is being discussed in very many journals and whose proposers may be recipients of a future Nobel Prize—is that it provides for multiple regulators ("operators" in the Jacob-Monod terminology) for the same gene. This means that other sensor genes can also switch on the synthesis of the same enzyme. It provides, in other words, for multiple links resembling a network. Many of you may still be wondering why should there be multiple regulators at the start of every producer gene? The answer would be more clear in the next figure.

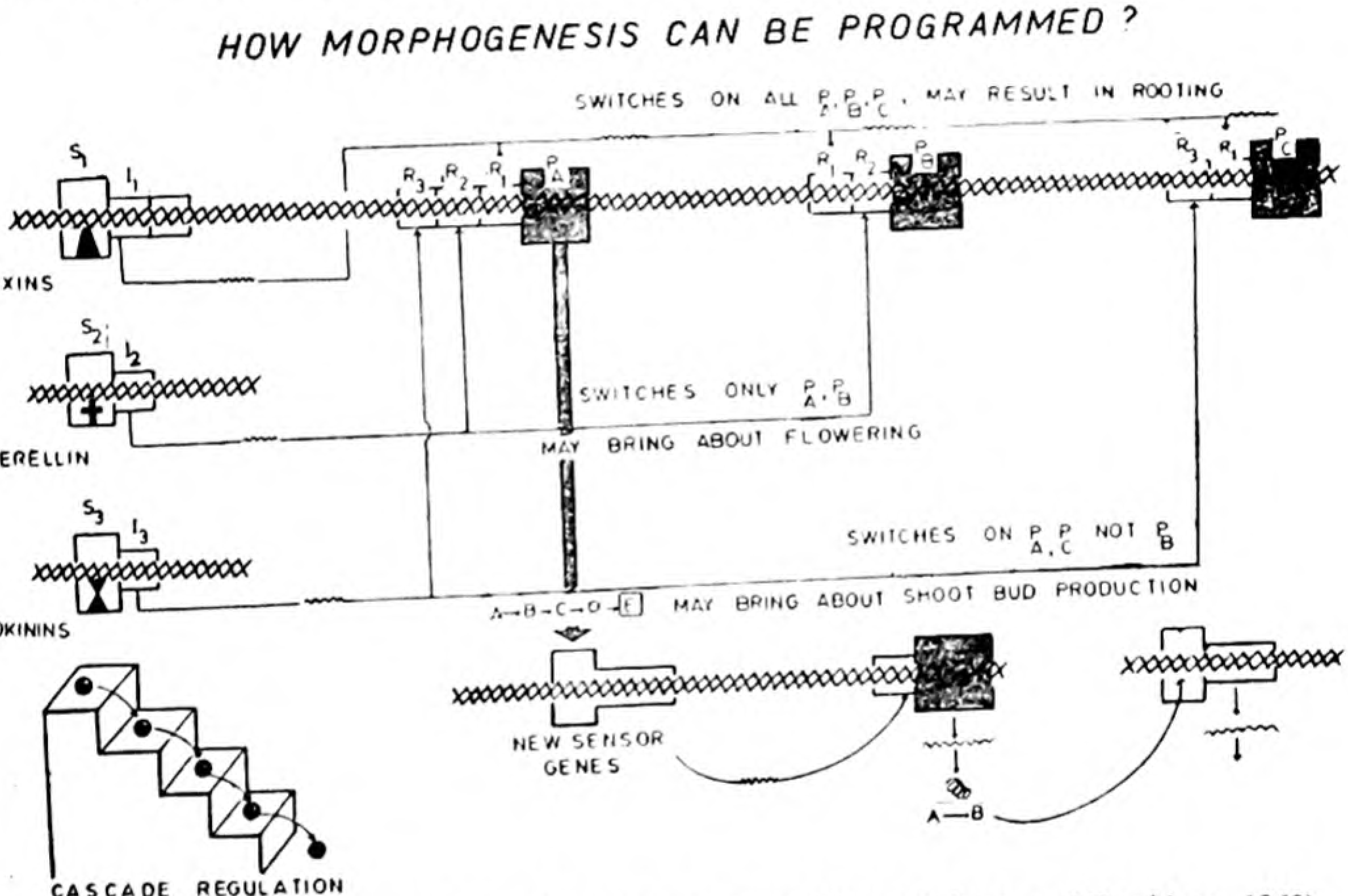
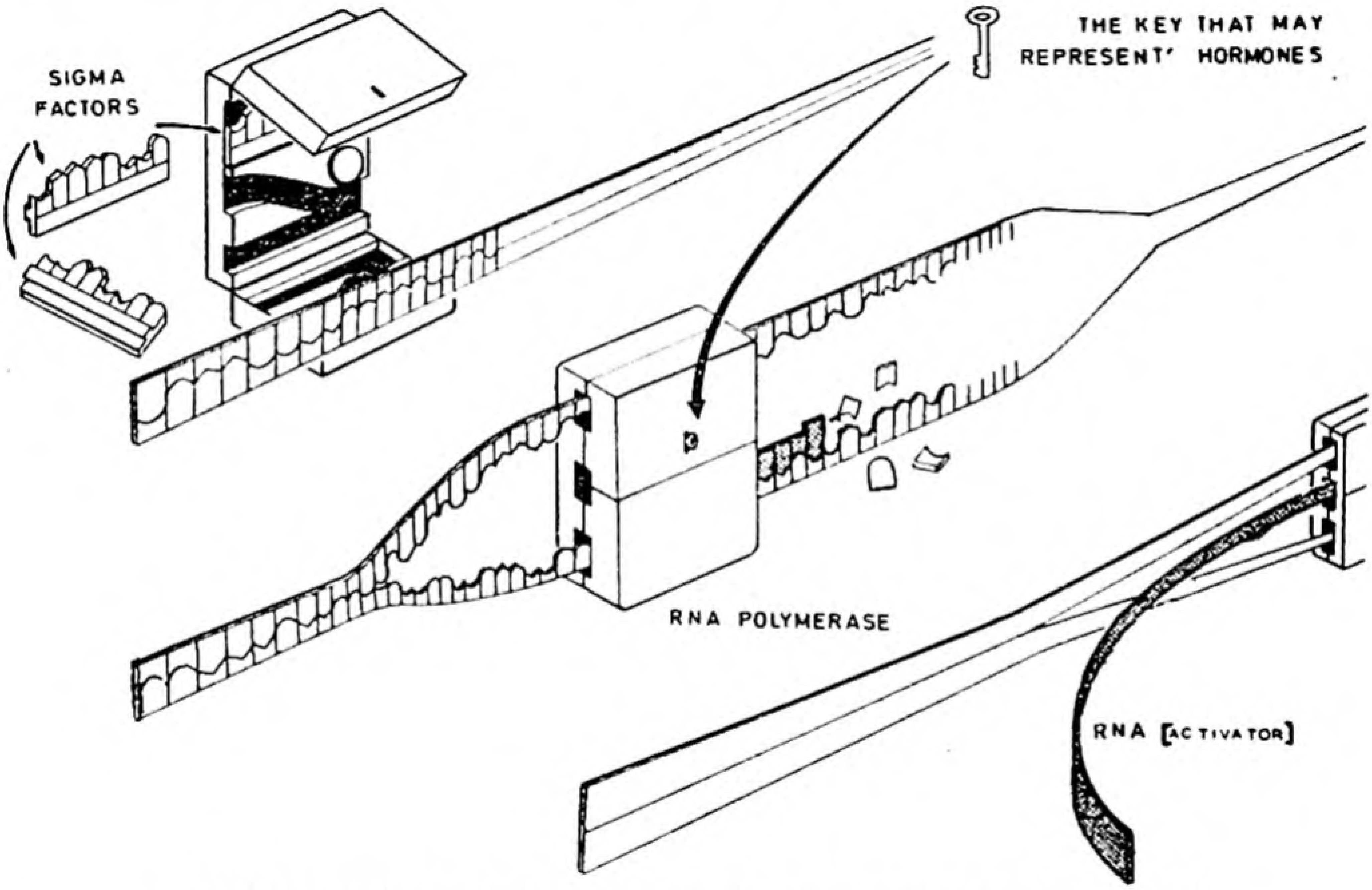

HOW MORPHOGENESIS CAN BE PROGRAMMED ?
SWITCHES ON ALL P_A, P_B, P_C, MAY RESULT IN ROOTING
S_1
I_1
R_3 R_2 R_1 P_A
R_1 R_2 P_B
R_3 R_1 P_C
AUXINS
S_2
I_2
GIBBERELLIN
SWITCHES ONLY P_A, P_B
MAY BRING ABOUT FLOWERING
S_3
I_3
CYTOKININS
SWITCHES ON P_A, P_C NOT P_B
A→B→C→D→E MAY BRING ABOUT SHOOT BUD PRODUCTION
NEW SENSOR GENES
A—B
CASCADE REGULATION

FIG. 7. The programming of morphogenesis (adapted from Britten and Davidson, 1969).

Figure 7 is the key to the entire theme. The point is that if you have multiple regulators—unlike the case in simple operons of bacteria—then a single sensitizing hormone or, here, at a more immediate level an activator RNA, can trigger switching on of hundreds of genes—all those which have at least one regulator corresponding to the sequence of bases of the integrator gene or the activator RNA. You have to appreciate that nearly all cells, whether they be in roots, stems, or leaves, have probably the same total number of active genes—40, 50, or 100, out of 1000-2000 or more that the cell may be endowed with. However, the permutations and combinations are different. That is exactly what onset of flowering, initiation of rooting, or budding demand. Let us follow this in a little more detail. An RNA coming from any one of these *integrators* will activate a producer gene provided there is one corresponding regulator sequence attached to it. Let us state that the nucleotide sequence of I_1 is similar to that of R_1, of I_2 to R_2 and of I_3 to R_3. Let us suppose that somehow the sensor gene S_1 gets activated—this would in turn activate I_{1A} and RNA I_1 will switch on producer genes P_A, P_B, and P_C, all of which have R_1 as one of their regulators. However, if sensor S_2 is sensitized, only P_A and P_B genes will get activated, and if sensor S_3 is activated then only P_A and P_C will get activated. As a matter of fact, depending on how many genes share R_1 (or for that matter R_2, R_3 etc.) a single sensor, through just one primary event may activate not merely one, two, or three, but huge batteries of dozens or even hundreds of producer genes. In all probability, this is how the auxins, gibberellins, and cytokinins, may trigger new morphogenetic development. Let us also not forget that the products of some of these activated genes may themselves sensitize a new set of sensors, and may thus turn on an entire new battery of genes. Such is the way in which differentiation and morphogenesis can be programmed, once a primary event has been triggered. Appropriately, this kind of



HOW HORMONES CAN TURN ON SENSOR GENES ?

FIG. 8. A model that explains how sensor genes, hormones and an RNA polymerase may work.

a control has been called *cascade regulation* (Waddington, 1962).

The next two figures (Figs. 8, 9) show in some detail as to how the components of the model may work. First, let us see how the sensor itself may work, (Fig. 8). One likelihood is that the sensor gene is the stretch of DNA which accepts a special RNA polymerase that works only in response to a hormone. There is evidence from several laboratories that RNA polymerase does consist of several parts (Burgess, 1971). The enzyme may be so constructed that in order to fit appropriately it may require, firstly, one of the sigma factors and to close secondly, it may require a key, I hope it is not difficult to see how these models may be fairly close to reality. In more chemical terms, closer to real life, it means that there is again a conformational change in the RNA polymerase—possibly from an open to a closed compact type. There is some evidence that at least a few of the hormones may function this way. For example, oestradiol in animals (Dati and Maurer, 1971) and auxins in plants (Venis, 1971) have been reported to bind to chromatin or its associated proteins. The key or the hormone, then, perhaps starts a special polymerase and the latter perhaps proceeds on briskly to synthesize the activator mRNA.

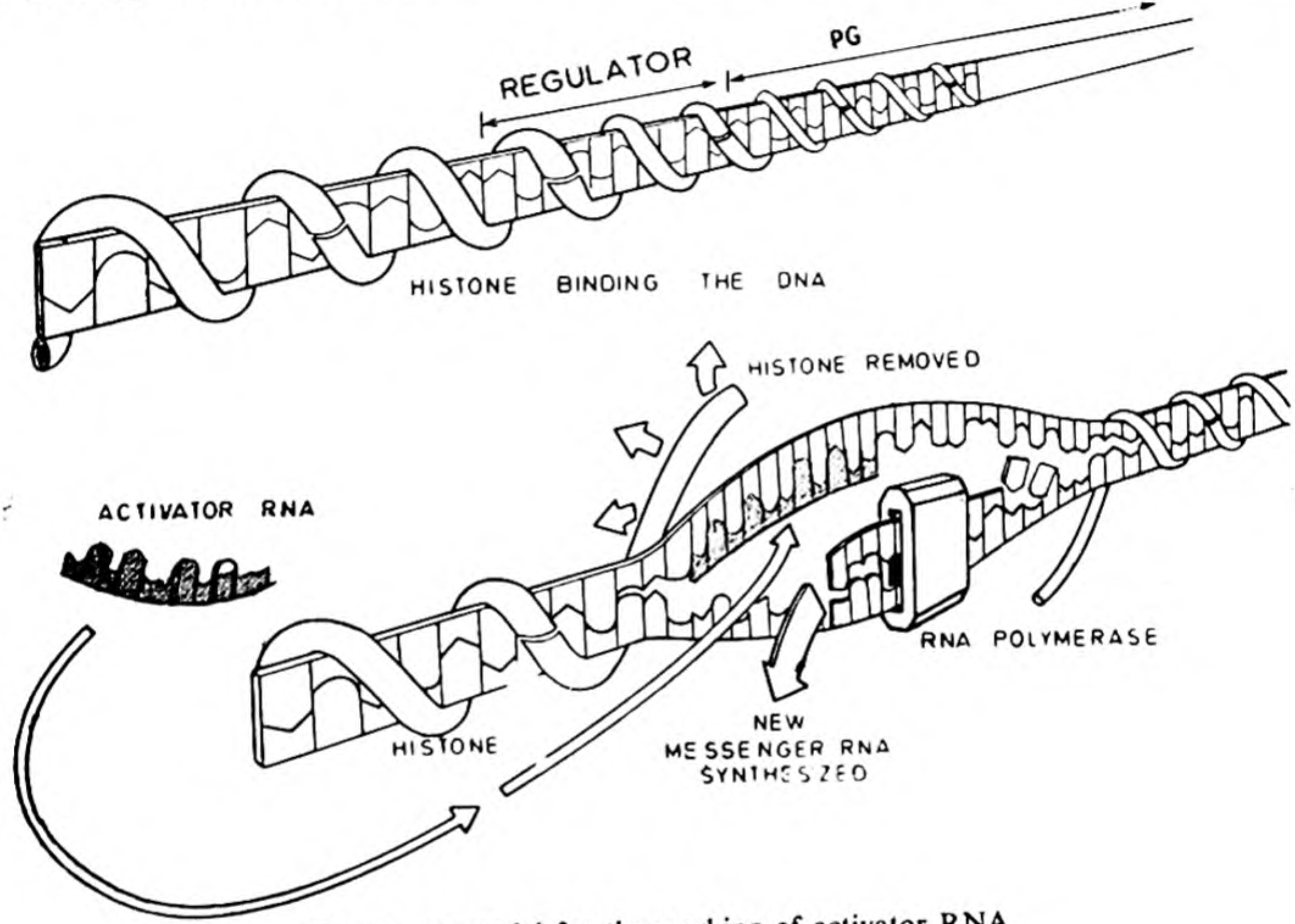


FIG. 9. A model for the working of activator RNA.

Figure 9 illustrates how the activator mRNA may switch on producer genes. The coiled components here represent histones which seem to normally keep all genes locked up in the higher organisms. The activator RNA may probably function by loosening or uncoiling the DNA helix by hydrogen-bonding with the antisense strand of the R gene. With one strand loosened and free, the contiguous producer gene may now be ready to be transcribed and produce messengers. Of course, there may be many copies of the same activator RNA and they would in real life activate not one but many genes. Those to whom this model is new, must be wondering as to how much of what I have mentioned is fact or fiction. The truth is that at present only the barest outline of this model is available through experiments done so far. Some of the world's most active laboratories, namely of Bonner at CalTech, Mirsky at Rockfeller University, Britten at Carnegie, Sidney Penman at MIT, John Paul at Glasgow, the MRC units of Molecular Biology at Cambridge and Edinburgh and others are engaged in research on one or other aspects of chromosome biology. There are many others who are shifting from the bacteria to the higher organisms and all this, to my mind, signifies a major effort in the *new* morphogenesis, which will become the most important theme of biology in the next two decades. Newer approaches are constantly being made. It is conceivable that the haploids, too, may be of great use in determining whether controls such as in bacteria exist in higher organisms. For example, one could, perhaps by the procedure shown in figure 10, map genes and determine whether or not polycistronic operons exist in the higher plants.

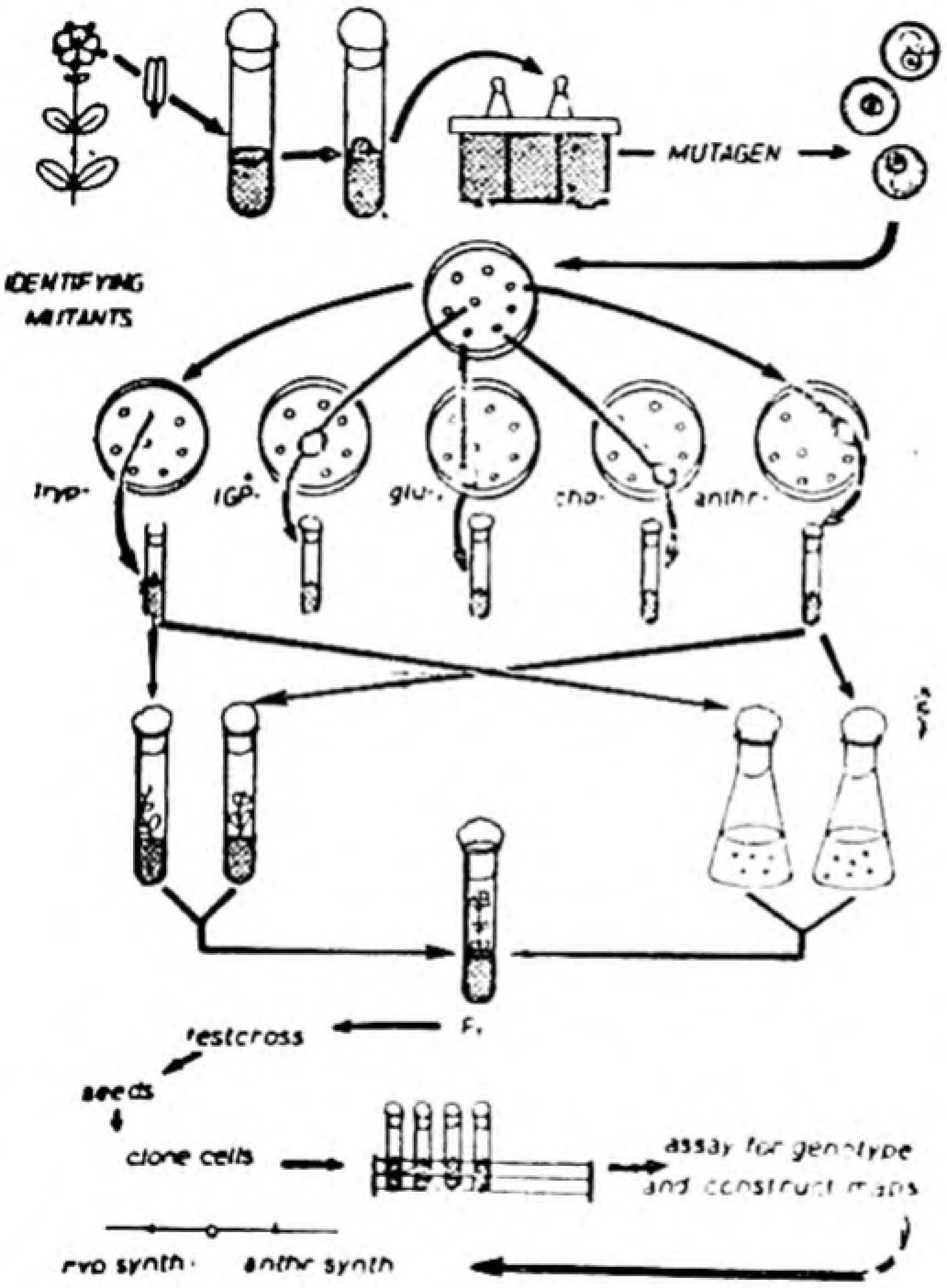


FIG. 10. An experimental design for the mapping of genes in higher organisms.

Figure 11 shows a mode of control of protein synthesis. It is text book knowledge that ribosomes are made up of one small and one larger subunit and that they bind to the messengers, initiating rounds of protein synthesis. The diagram illustrates a very important and recent finding (Lee Huang and Ochoa, 1971) that there exists another component called the *recognition* or *initiation* factor without which the assembly is incomplete. This component is so constructed that on the one hand it recognizes the subunits and, on the other, the starting sequence of a messenger RNA. There is growing evidence that in cells there are messengers with various types of leading recognition sequences and so also there are many types of initiation factors. Again, a further control may be provided by a small linker. Certain observations in the laboratories of Chen and Osborne (1970) at Cambridge and Berridge et al., (1970) in New Zealand, concerning the mechanism of action of cytokinins and gibberellic acid, in some systems, are explainable only in terms of some such idea. The cells may also somehow cause one initiation factor to abound more than others. And that would lead to new types of enzymes being made in a cell.

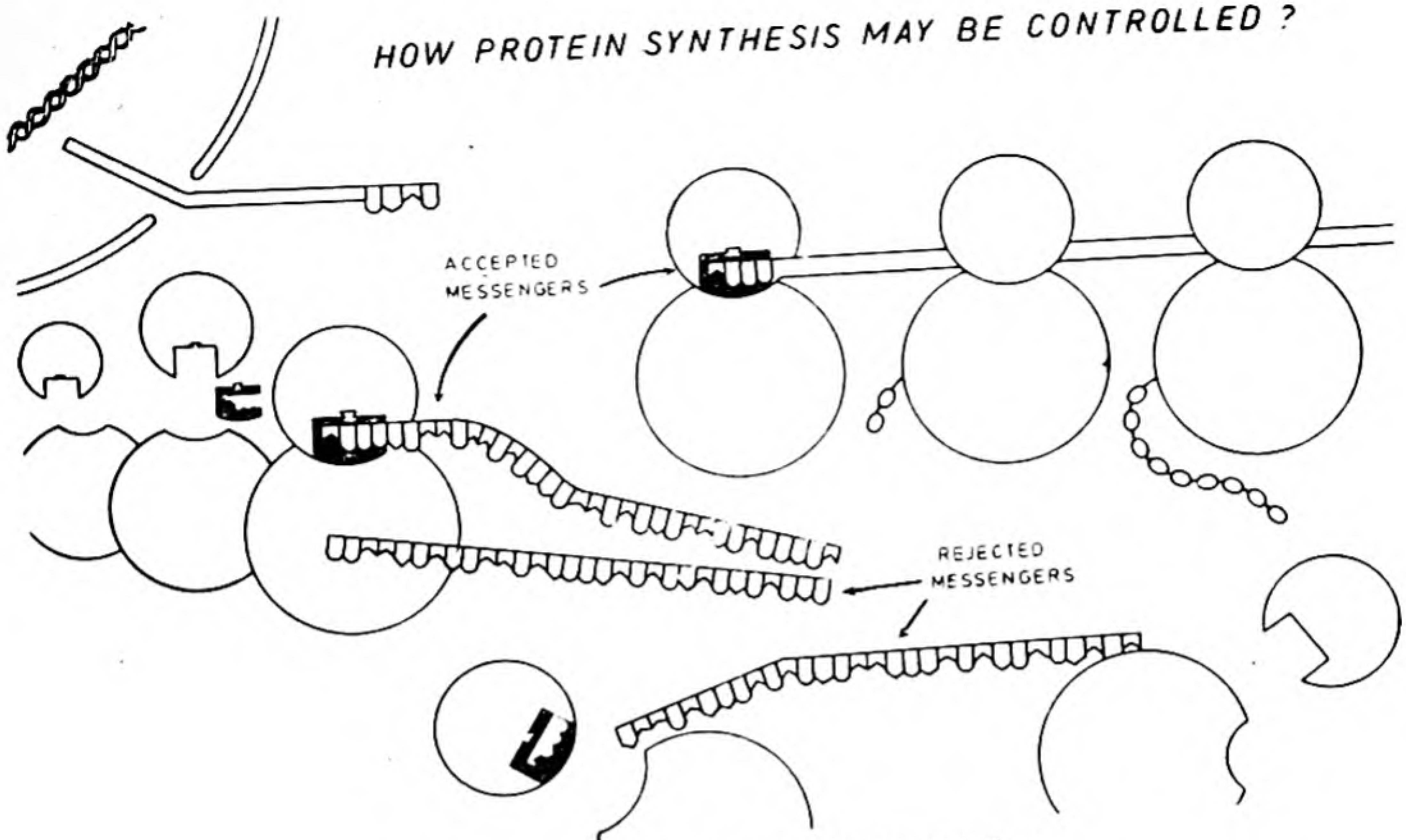

HOW PROTEIN SYNTHESIS MAY BE CONTROLLED ?
ACCEPTED MESSENGERS
REJECTED MESSENGERS

FIG. 11. A model for the control of translation.

Vital to ordered differentiation is also the control of activity of enzymes—those enzyme molecules already made and existing in the cytoplasm. This is shown in Fig. 12. There are many enzymes whose activity is regulated. These controllable enzymes happen to be usually those that are at the start of a biosynthe-

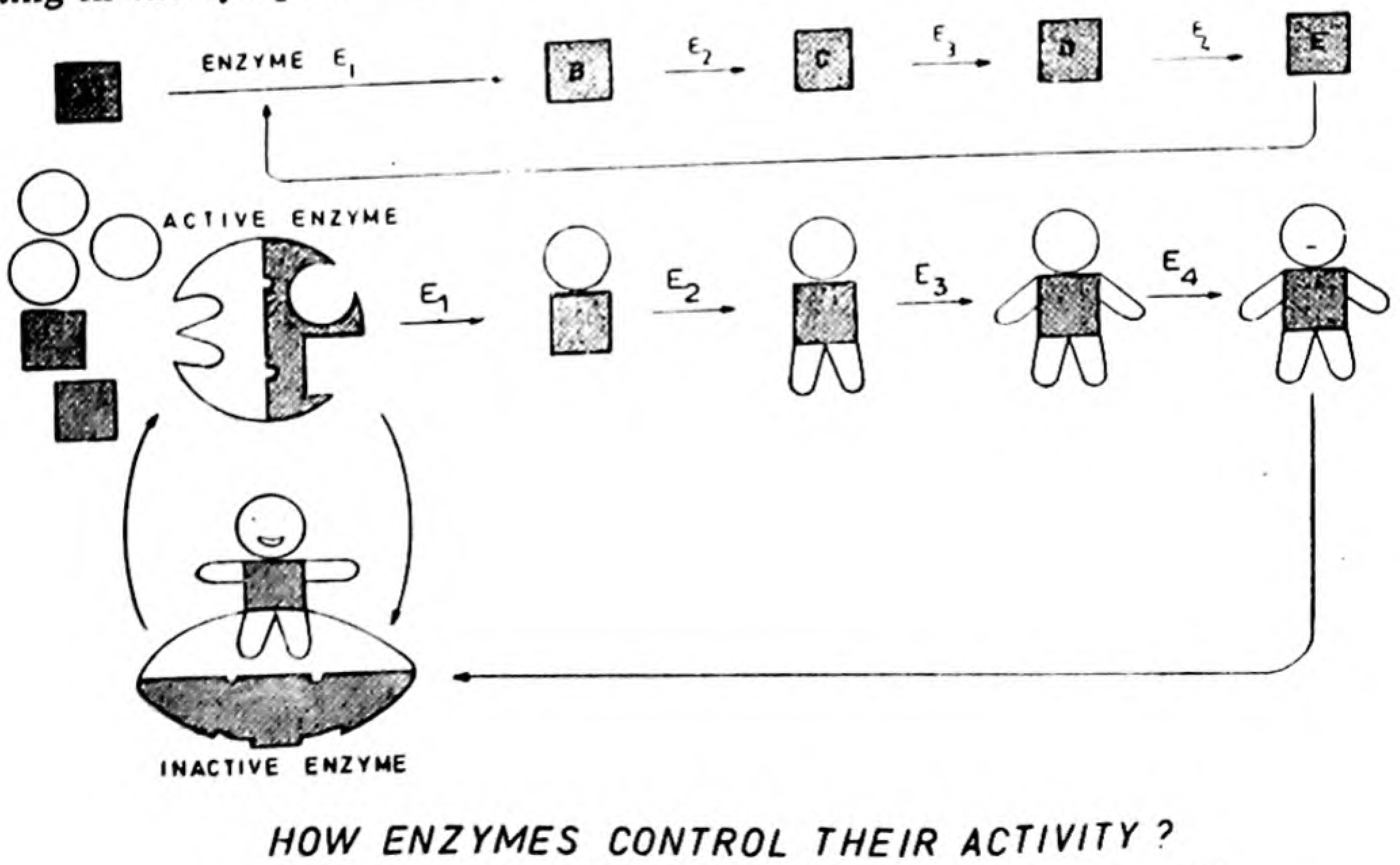

ENZYME E1
B
E2
C
E3
D
E4
E
ACTIVE ENZYME
E1
E2
E3
E4
INACTIVE ENZYME
HOW ENZYMES CONTROL THEIR ACTIVITY ?

FIG. 12. Feed back mechanism for the regulation of enzymatic activities.

tic pathway such as the one hypothesized here— if the activity of the end product is in excess you certainly need a method of regulating the activity of the enzyme. Under certain different circumstances you may need to activate a resting enzyme. Now it so happens that these enzymes are complex and are made up of more than one subunit—at least one catalytic and the other regulatory (Gerhart et al., 1965). The regulatory part is so constructed that the end product can fit into it and the conformation of our enzyme can be altered in such a way that it no longer works. Of course, such binding will come about only if the number of the end product molecules (little men in fig. 12) exceeds a certain critical because basically it is a probability affair. My reason for illustrating this mechanism of enzyme control is that already there is definite evidence that small molecules, other than substrates, also alter enzyme activity. While attachment of end-product leads to inhibition, that of certain other types of molecules leads to a better conformation and activation. In fact, it appears that a large number of enzymes essential to growth and differentiation are activated by the recently discovered hormone, cyclic AMP (Jost and Rickenberg, 1971).

We shall now consider the mechanism of action of phytochrome—the pigment that is responsible for regulation of plant growth by light. This is one of the recent and outstanding discoveries of plant science (Butler et al., 1959). Light affects many processes in the plant, in a manner other than through photosynthesis and I trust that every botanist must have, sometime or the other, seen the formulation in Fig. 13 representing interconversions between the two forms of phytochrome—the *red* and the *far-red* absorbing forms—by red and far-red light, or darkness. Many in this audience may wonder what these symbols represent and what is the real nature of change between P_R and P_{FR} forms. The lower part of Fig. 13 shows a model that may come out to be true in the next decades. According to some current ideas, phytochrome may represent an enzyme or a set of enzymes controllable by light (NATO), 1971; Hug et al., (1971). So far, we have known of regulation of enzyme activity, i.e., the changes from active to inactive configuration or vice versa only in response to attachment of little molecules—end products, cyclic AMP etc. But a novel idea, developing among workers in this field, is that conformational changes may take place only in response to molecules but even photons—say of red light. The enzyme could contain a chromophore which, as it undergoes the cis-trans conformational change, also simultaneously alters the shape of the protein to which the chromophore is attached. Already, evidence is accumulating that several enzymes, e.g., PEP-carboxylase, on which

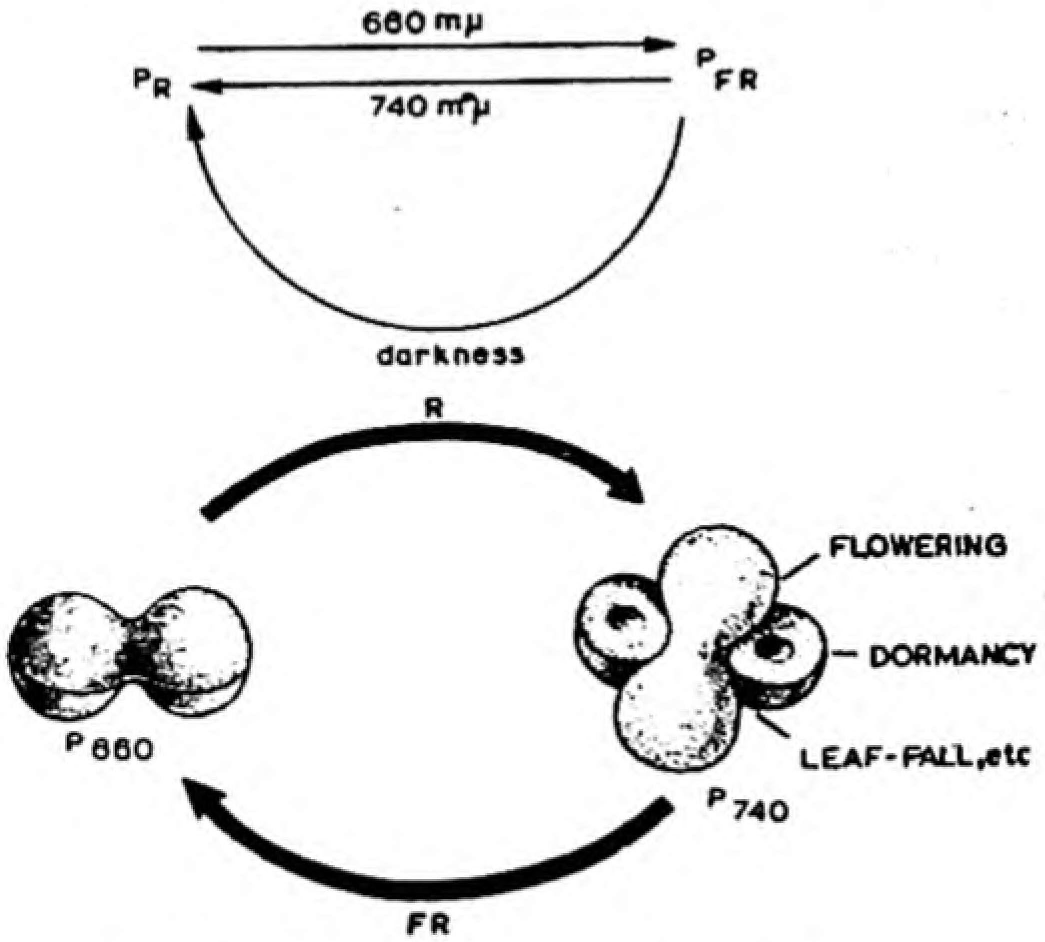


FIG. 13. How light can alter conformation of phytochrome molecules—a model.

some work is being done also in our laboratory, may be a light-controlled enzyme (Marcus, 1971). The future may see phytochrome resolve out into one or more such identifiable enzymes. These enzymes could, of course, exist either freely in the cytoplasm, or may be bound to membranes as integral components.

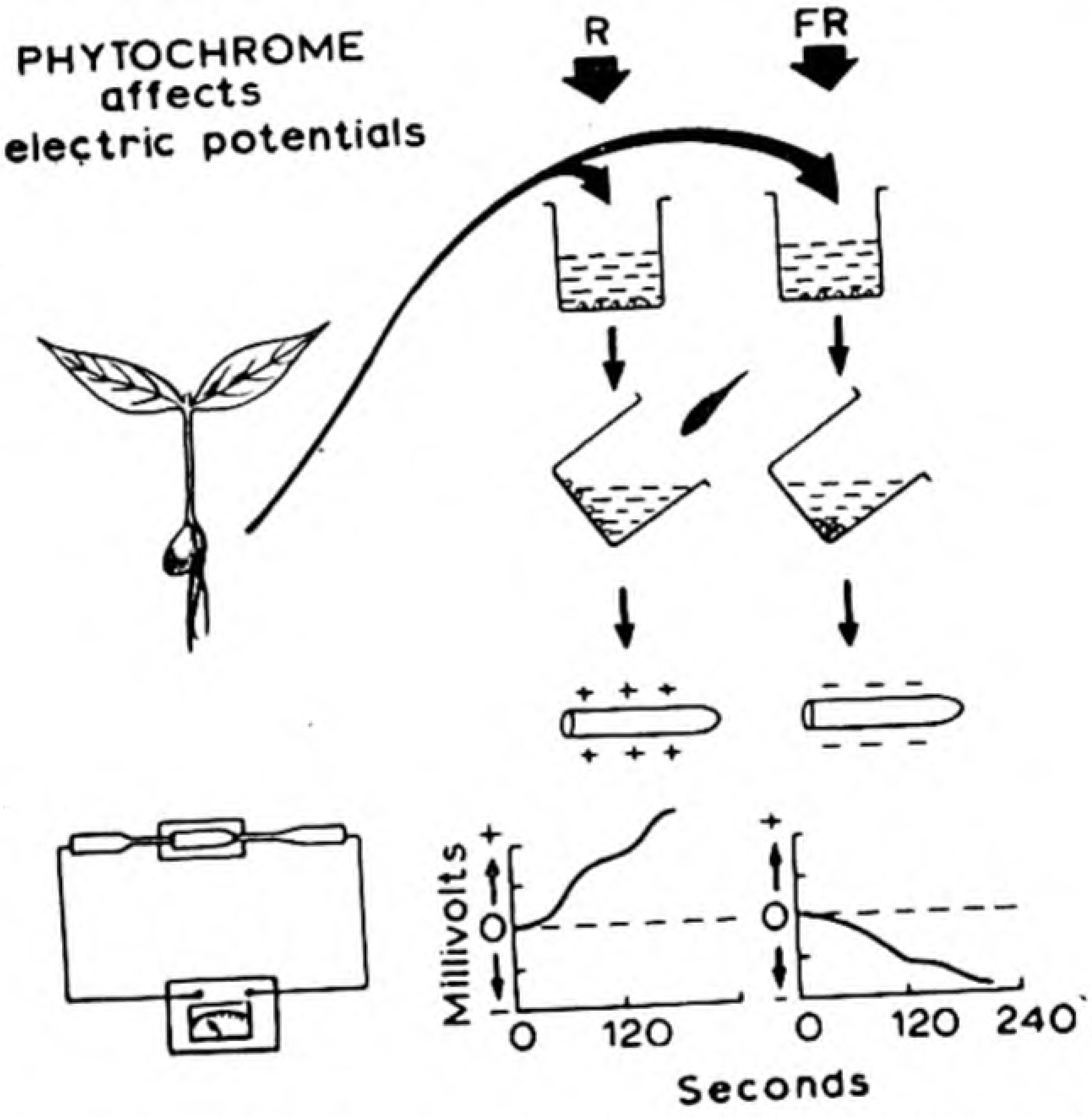


FIG. 14. Diagram to illustrate effects of red and far-red light on bean root tips.

Figure 14 brings us to another exciting finding, the possible introduction of biophysics in the study of morphogenesis in a bold and strident manner. It is well known that understanding of the working of the brain and the nervous system has depended a lot on biophysical work, such as being done by Professor Paintal in my university. In a very intriguing paper, a claim has recently been made that red and far-red light can control the charge on the membrane (Tanada, 1968). Excised root tips of mung bean stick to the glass surface (which is negative) in red light and detach in far-red light. Refined electrical measurements by use of high impedance microvoltmeters show that in the red light the root tips become positive—they develop within minutes a charge of about one millivolt—and in far-red light a negative charge of the same magnitude (Jaffe, 1968). The charges may explain many features of growth and differentiation, such as polarity, curvatures, etc. Indeed, a claim has been made that acetylcholine, the neurohormone present in nerve membranes, exists also in plants (Jaffe, 1970) and its level markedly changes upon R or FR iradiation (Fig. 15). It is remarkable too that acetylcholine has already been shown to affect profoundly growth and development in fungi (Gressel et al., 1971). If this is true also of higher plants, much work along these lines can be expected in the next 20 years on membranes—inspired, of course, by the great mystery of differentiation.

We must also appreciate that the membranes are hardly like the protein-lipid double layer model proposed by Davson and Danielli years ago. On the other hand, evidence has been accumulating (Green and Brucker, 1972) that it is particulate and consists of tiny subunits (Fig. 16). It is also clear that many of these subunits—far from being dead structural proteins—are active enzymes that participate in many essential tasks such as diffusion of ions. Again, it is clear already that iron, several sugars and amino acids do not just diffuse in but that there are specific permeases located in the wall which, so to speak, catch the respective molecules and shove them in. Many investigators believe that phytochrome is localized in membranes. Perhaps phytochrome may be a permease of some kind (Harry, 1971). You may conceive of the little men (permeases) reclining and not standing as shown, In the reclining posture they would not work and photons of light—like sunshine falling into the eyes—may "wake up" the permeases. All you have to do to design such enzymes is to attach a chromophore to the protein. It seems certain that in the next two decades much work will be done on membranes—whether chromosomes will get more attention or membranes is a bit difficult to predict.

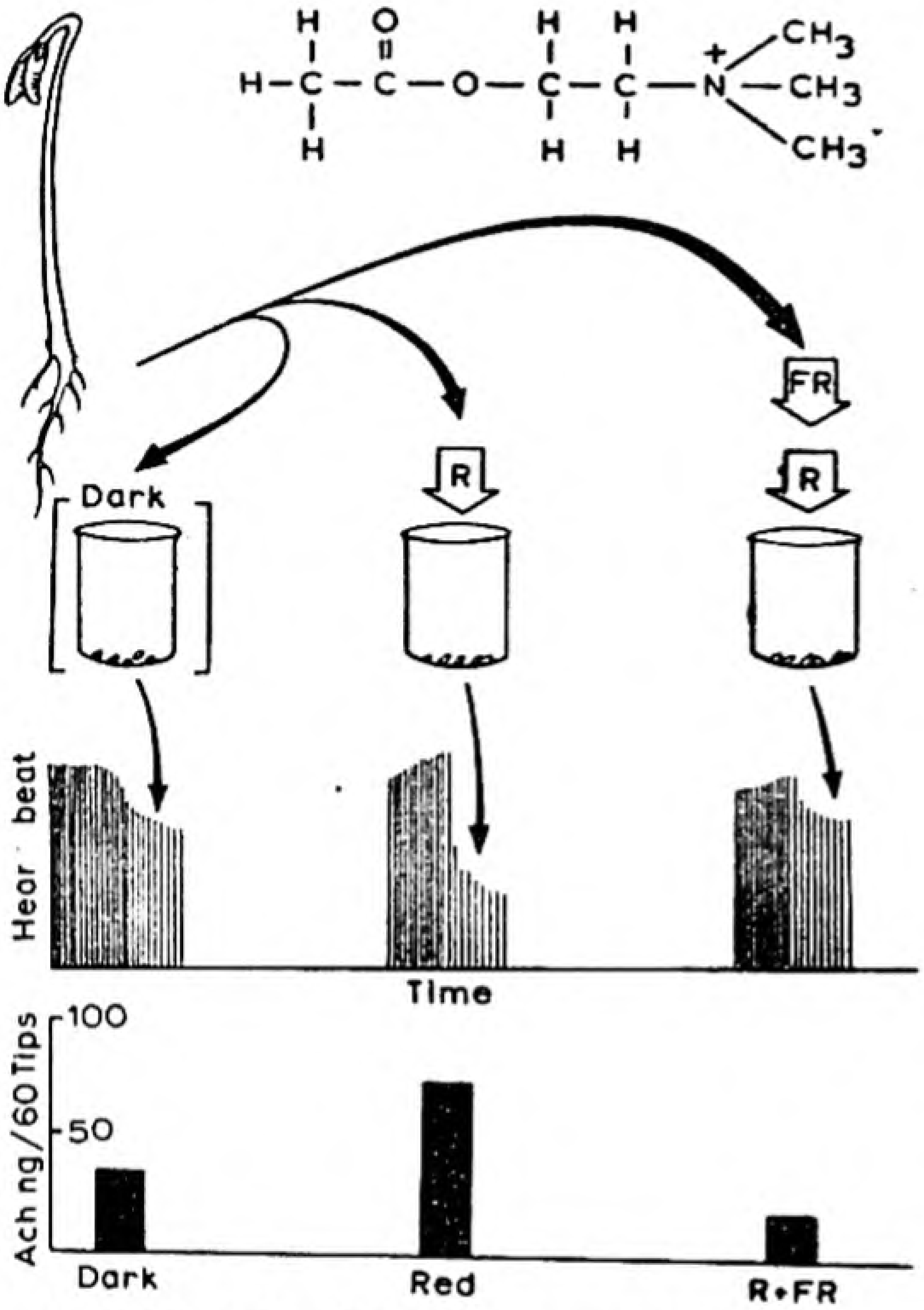


FIG. 15. The effect of red and far-red light on levels of acetylcholine in bean root tips (after Jaffe, 1970).

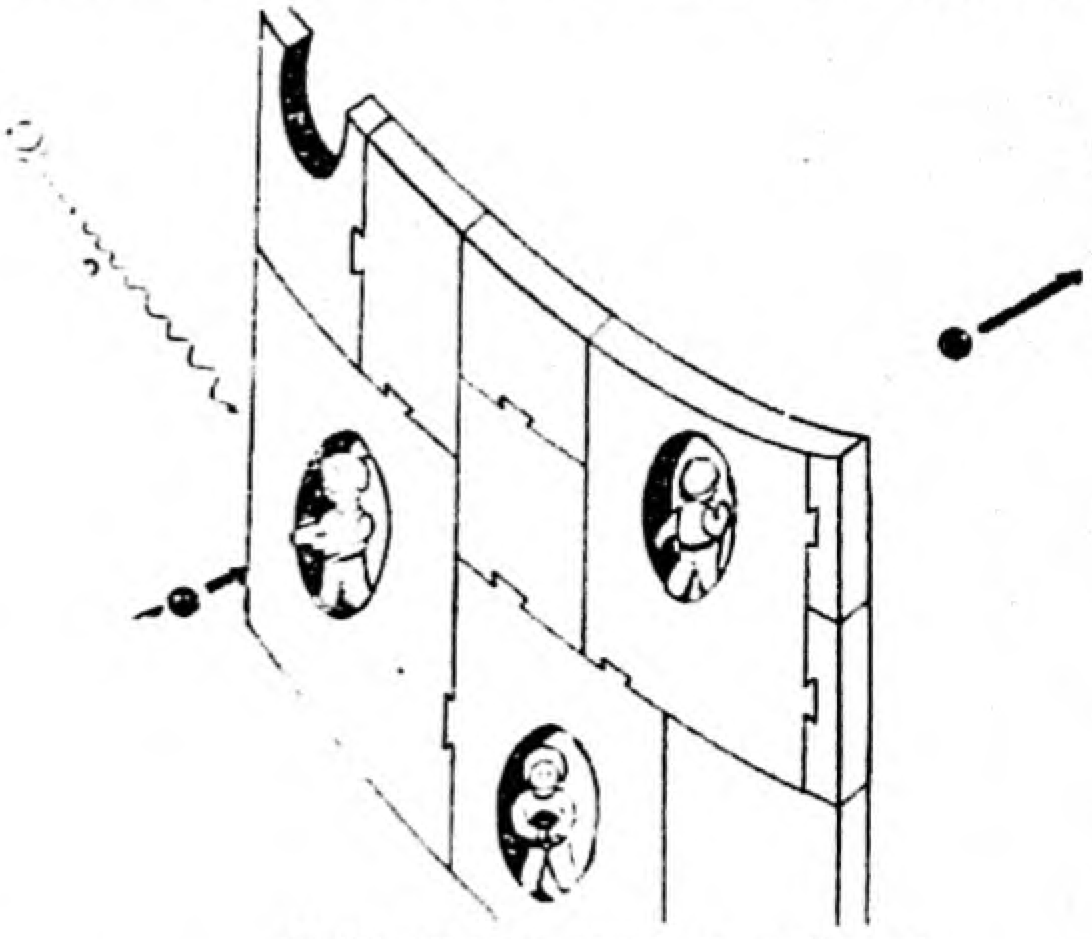
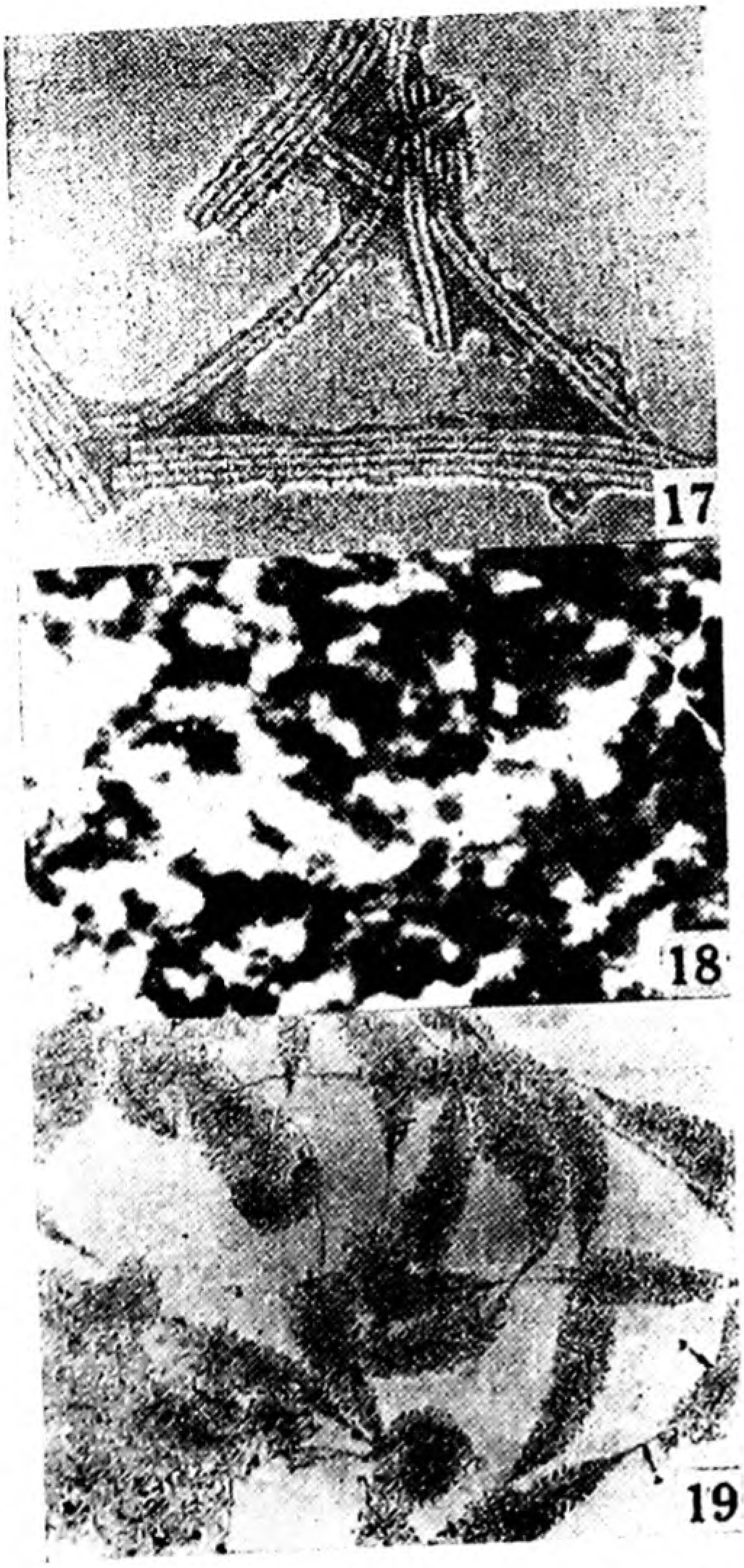

HOW MEMBRANES FUNCTION ?

FIG. 16. Model for the functioning of membranes.

FUTURE OF ELECTRON MICROSCOPY

The last three figures are concerned with the place of electron microscopy in future research in morphogenesis. Finding of new organelles and new structures certainly has a relevance to morphogenesis and differentiation. However, it is my estimate that the major concepts and contributions, as in the past, will come from biochemical techniques and will far outweigh those by electron microscopy. This is because of the inherent limitation of the electron microscope which cannot deal with living matter and cannot show things in a dynamic state.



FIGS. 17-19. Fig. 17, Rods of tobacco mosaic virus. Fig. 18. Pea DNA under an electron microscope. Fig. 19. Ribosomal genes from oocyte of newt (after Miller and Beatty, 1969).

According to Huxley and Klug (1971) of the MRC Laboratory, Cambridge, who recently organized a conference on the future of electron microscopy under the auspices of the Royal Society—the days of classical electron microscopy are over. New organelles like ribosomes and mitochondria are not likely to be discovered. The future is really in the high resolution field—structures less than about 15A° diameter—and it is through this kind of high resolution work that many of the concepts outlined here may be confirmed by electron microscopy. However, a point I would like to make is that high resolution microscopes are nearly, if not totally, useless with sections. Even the thinnest sections are generally too thick to get a resolution of even 7 and 8 A°, let alone 3 or 5 A°. Figure 17 shows the picture of TMV rods—note the high resolution, and this is not a section. In figure 19 one sees the marvellous picture of ribosomal genes—ribosomal RNA is being synthesized and cistrons are clearly visible—but this too is not a section. Lastly, there is the astonishing picture (Fig. 18) of what is believed to be the two helices of DNA—and this too is not a section. Many laboratories are trying to obtain electron micro scopes and we have several in the country. However, in order to properly use the high resolution capability the organelle or molecule must generally be isolated from the morass of the whole cell—which requires biochemical procedures. If such techniques are followed, the activator RNA, histones etc, might be seen—such work is already being done and that is where the future of electron microscopy is.

CONCLUSION

Certainly it seems to me that morphogenesis has a very bright future. I make two predictions. First, that the coming two decades will see a fusion of the various specialities of biology—genetics, microbiology, biochemistry and biophysics—all of which eventually will converge on the same point, morphogenesis, and we shall soon see the mystery lift from the most baffling of the present problems of biology. Time and space do not permit me to mention all possible directions of future advances—for considerations of clarity too only a few main themes could be developed here. However, we must recognize that there are such directions —to mention one, identification of new growth hormones.

My fervent hope is that at least one or two universities in India will organize the kind of effort for doing the *NEW* morphogenesis and enable the younger generation to experience the excitement of pure science. Of course, such work will require a good deal of hard work and dedication. In fact, my objective is presenting this lecture will not be realized if I fail to mention that in order to participate in the endeavour to do this new morphogenesis, we shall have to familiarize the students of tomorrow with the new tools of molecular biology and biochemistry, tools such as for separation of cell organelles, of chemical compounds, for measurement of chemical changes in cell extracts and cells themselves, and determination of redioactivity. In order to understand the language of new morphogenesis it may become necessary also to familiarize the students with physics and the language of mathematics. And certainly to accomplish this we shall have to be prepared to familiarize ourselves, first, with the new developments. Only then, perhaps we shall be able to meaningfully contribute to the advent of the new morphogenesis.

I wish to acknowledge that this paper, could not have been completed without the invaluable assistance of two of my present Ph. D. students, Mr. S. K. Sopory and Miss S. Lakshmi. My gratitude is also due to my other previous students, Drs S. Mukherjee, M.M. Johri, P.N. Seth, P.R. Bhalla, R. Venkataraman, Ritu Prakash, Renu Anand, Subhashini Gupta, and Geeta, R.P., for stimulating discussions over many years.

REFERENCES

ADDICOTT, F. T., AND J. L. LYON. 1969. Physiology of abscisic acid and related substances. *Ann. Rev. Pl. Physiol.* **20:** 139-164.

BERRIDGE, M. V., R. K. RALPH, AND D. S. LETHAM. 1970. The binding of kinetin to plant ribosomes. *Biochem. J.* **119:** 75-84.

BRITTEN, R. J., AND R. H. DAVIDSON. 1969. Gene regulation for higher cells: A theory. *Science* **165:** 349-358.

BURGESS, R. 1971. RNA polymerase. *Ann. Rev. Biochem.* **40:** 711.

BUTLER, W. L., K. H. NORRIS, H. W. SIEGELMAN, AND S. B. HENDRICKS. 1959. Detection, assay and preliminary purification of the pigment controlling photoresponsive development of plants. *Proc. Nat. Acad. Sci. U.S.A.* **45:** 1703-1708.

CHEN, DAVID, AND DAPHNE J. OSBORNE. 1970. Hormones in the translational control of early germination in wheat embryos. *Nature Lond.* **226-:** 1157-1159.

DARWIN, C. 1880. *The Power of Movement in Plants.* Appleton Century Crofts Inc, New York.

DATI, F. A., AND H. R. MAURER. 1971. Estradiol mediated template activity of mouse and rat chromatin: an unsolved problem. *Biochem. Biophys. Acta.* **244:** 589.

GARNER, W. W., AND H. A. ALLARD. 1920. Effect of the relative length of day, night and other factors of the environment on growth and reproduction in plants. *J. Agric. Res.* **18:** 553-606.

GERHART, JOHN C., AND HOWARD, K. SCHACHMAN. 1965. Distinct subunits for the regulation and catalytic activity of aspartate transcarbalylase. *Biochem.* **4:** 1054.

GREEN, D. E., AND R. F. BRUCKER. 1972. The molecular principles of biological membrane construction and function. *BioScience* **22:** 13.

GRESSEL, J., L. STRAUSBAUCH, AND E. GALUN. 1971. Plant growth photomimetic effect of acteylcholine. *Nature, Lond.* **232 (5313: 648-649).**

Guha, S., and S. C. Maheshwari. 1964. In Vitro production of embryos from anthers of *Datura*. *Nature*, *Lond*. **204:** 497.

Haberlandt, G. 1902. Kulturversuche mit isolierten Pflanzenzellen. *Sitzungber. Acad Wiss. Wien. Math-naturw. Kl. III*: 69-92.

Hamner, K.C., and J. Bonner. 1938. Photoperiodism in relation to hormone as factors in floral initiation. *Bot. Gaz.* **100:** 388-431.

Harry, Smith. 1971. Phytochrome and photomorphogenesis in plants. *Nature, Lond.* **227:** 665.

Hendricks, S. B., and H. Borthwick. 1959. Photocontrol of plant development by simultaneous excitations of two interconvertible pigments. *Proc. natn. Acad. Sci. U.S.A.* **42:** 19-26.

Hug, D. H., D. Roth., and J. K. Hunter. 1971. Photoactivation of an enzyme and biological photoreception: a hypothesis. *Physiol. Chem. Phys.* **3:** 353.

Huxley, H. E., and A. Klug. 1971. A discussion on new developments in electron microscopy with special emphasis on their application in biology. *Phil. Trans. Royal Soc. London. Biol. Sci.* **261:** 221.

Jacob. F., and J. Monod. 1961. Genetic regulatory mechanisms in the synthesis of proteins. *J. Mol. Biol.* **3:** 318.

Jacobson, Ralph A., and J. Bonner. 1971. Studies of the c-RNA and of the c-RNA-binding proteins of higher organisms. *Arch. Biochem. Biophys.* **146:** 557.

Jaffe, M. J. 1968. Phytochrome mediated bioelectric potentials in mung bean seedlings. *Science, Wash.* **162:** 1016.

——. 1970. Evidence for the regulation of phytochrome mediated processes in bean roots by the neurohumor, acetylcholine. *Pl. Physiol.* **46:** 768–777.

Jost, J. P. and H. V. Rickenberg. 1971. Cyclic AMP. *Ann. Rev. Biochem.* **40:** 741.

Kogl, E., A. J. Haagen-smit, and H. Erzleben. 1934. Uber ein neues Auxin ("Heteroauxin") aus Harn. XI. Mitteilung *Z. Physiol. Chem.* **228:** 90-103.

Kusum Kanta, N. S. Rangaswamy, and P. Maheshwari. 1962. Test-tube fertilization in a flowering plant. *Nature, Lond.* **194:** 1214-1217.

Lee-Huang, and S. Ochoa. 1971. Messenger discriminating species of initiation factor F_3. *Nature New Biol.* **234:** 236.

Letham, D.S. 1963. Purification of factors inducing cell division extracted from plum fruitlets. *Life Sciences* No. 3: 152-157.

Mac Millan, J., J. C. Seaton, and P. J. Suter. 1961. Isolation and structures of gibberellins from higher plants. *Adv. Chem. Ser.* **28:** 18-25.

Marcus, Abraham. 1971. Enzyme induction in plants. *Ann. Rev. Pl. Physiol.* **22:** 313.

Melchers, G. 1972. Haploid higher plants from pollen. *Zeit. Pfl Zücht.* **67:** 19-32.

Nato. 1971. Advanced study institute on phytochrome. *Nature, New Biol.* **233:** 159.

Phinney, B. O., West, C. A., M. Ritzel, and P. M. Nealy. 1957. Evidence for 'gibberellin-like' substances from flowering plants. *Proc. natn. Acad. Sci. U.S.A.* **43:** 398-404.

Power, J. B., Cummins, and E. C. Cocking, 1970. Fusion of isolated plant protoplasts. *Nature, Lond.* **225:** 1016-1018.

Pratt, H. K., and J. D. Goeschl. 1969. Physiological roles of ethylene in plants. *Ann. Rev. Pl. Physiol.* **20:** 541-581.

Steward, F. C. 1959. Interpretation of the growth from free cell to carrot plant. *Am. J. Bot.* **45:** 709-713.

Sivolap, Yuri M., and J. Bonner, 1971. Association of c-RNA with repetitive DNA. *Proc. Nat. Acad. Sci. U.S.A.* **68:** 387-389.

Tanada, T. 1968. A rapid reversible response of barley root tips in the presence of 3-indoleacetic acid. *Proc. natn. Acad. Sci. U.S.A.* **59:** 376.

Van Overbeek, J , M. E. Conklin, and A. F. Blakeslee. 1941. Factors in coconut milk essential for growth and development of very young *Datura* embryos. *Science, Wash* **4:** 350-351.

Venis, M.A. 1971. Stimultion of RNA transcription from pea and corn DNA by protein retained on Sepharose coupled to 2, 4—dichlorophenoxy acetic acid. *Proc. natn. Acad. Sci. U.S.A.* **68:** 1824-1827.

Waddington, C.H. 1962. *New Patterns in Genetics and Development*. Columbia Univ. Press, New York.

Wareing, P. F., J. Good, and J. Manuel. 1968. Some possible physiological roles of abscisic acid. pp 1561-1579. In *Biochemistry and Physiology of Plant Growth Substances*. F. Wightman and G. Setterfield (ed.) Runge Press, Ottawa.

White, P.R. 1934. Potentially unlimited growth of excised tomato root tips in a liquid medium *Pl. Physiol.* **9:** 585-600.